PHD

The design, synthesis and pharmacological evaluation of ligands targeted at the kappa opioid receptor

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The Design, Synthesis And Pharmacological Evaluation Of Ligands Targeted At The Kappa Opioid Receptor

submitted by Shannon Leigh Black

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ABSTRACT

κ-Agonists are no longer investigated for the treatment of pain, however there has recently been significant interest in the role of κ-opioid agonists in cocaine abuse, alcoholism and the regulation of food intake. There is thus an interest in κ-selective antagonist compounds both as pharmacological tools, to probe the function of the κ-opioid receptor, and as potential therapeutic agents.

The κ-selective antagonist presently in use is norBNI. The pharmacological profile of this bivalent ligand is however complicated due to poor solubility, slow onset and extremely long duration of action. Studies have shown that the second pharmacophore is not essential, but rather, it is the presence of the second basic nitrogen that is crucial in retaining good κ-antagonist selectivity. It was believed that lower molecular weight, less lipophilic ligands would show an improved pharmacological profile. The synthesis of a variety of 5'-amino- and amidinosubstituted naltrindole analogues has afforded compounds displaying good κ-selectivity and efficacy. By further probing the optimum distance between the two basic centres, as well as the level of basicity required, we sought to develop ligands with increased selectivity. We therefore synthesised further series of 5'-amido, amidino, imidazoline and urea substituted compounds for pharmacological evaluation. The highly basic amidino and imidazoline compounds displayed good affinity for the κ-receptor. The affinity was somewhat lower for the non-basic amide compounds. The urea derivatives were slightly δ-selective, a finding attributed to an interaction between a basic residue in the δ-receptor and the C=O of the urea moiety. The most highly κ-selective compound reported thus far is the 5'-guanidino-substituted, GNTI. Attempting to increase κ-selectivity, we prepared substituted analogues of GNTI in the hope that the increased bulk would decrease affinity for the μ- and δ- receptors.

In order to investigate the binding of the compounds to the κ-opioid receptor, they were docked into a SYBYL generated model. Limited selectivity studies were conducted by docking compounds into the μ-, δ- and κ-receptor models and comparing the differences. By investigating the interactions between the receptor and the ligands, we were able to explain some of the trends observed in pharmacological assays such as the high affinity of the amidines and the low affinity of the urea derivatives. In addition, we were able to propose further synthetic targets that may display good κ-selectivity and/or affinity.

ACKNOWLEDGEMENTS

Thanks to my supervisors John Lewis and Steve Husbands for advice and guidance. Special thanks go to Steve for going beyond the call of duty, particularly in transporting my belongings back and forth across the UK!

My time spent in the UK was made all the more enjoyable by sharing with great housemates. Thanks to all the members of the Richmond Terrace family, particularly Paola, Muk, Markus, Alex, Ed, Sanae, Federica and Marcellus, and also to Peter, Jazz and Tanja from 54 Winchester Rd.

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"Trust in the Lord with all your heart and lean not on your own understanding; in all your ways acknowledge Him and He will make your paths straight."

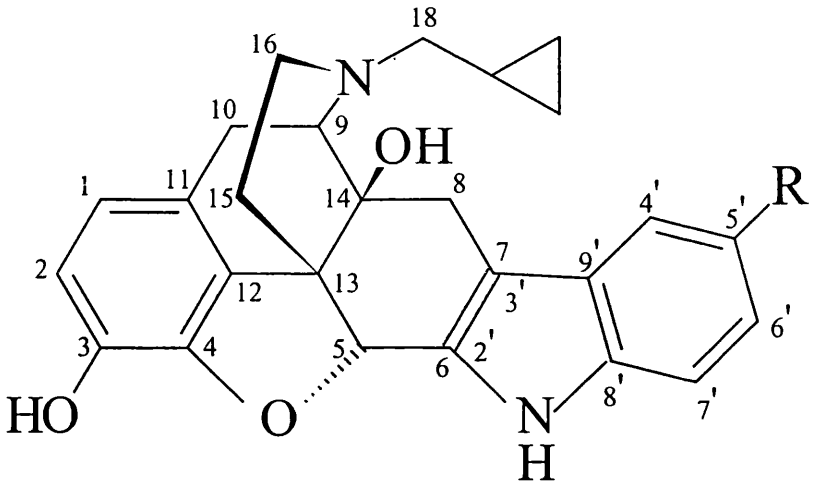
Pro. 3 : 5-6

CONTENTS

ABBREVIATIONS

BOC	*t*-butoxycarbonyl
BOP reagent	benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate
°C	degrees Celsius
δ	chemical shift (NMR spectra)
d	doublet (NMR spectra)
DEPT	distortionless enhancement by polarisation transfer
DIBAL	diisobutylaluminium hydride
DMF	*N,N*-dimethylformamide
DMSO	dimethylsulphoxide
EI	electron ionisation
eq	equivalents
FAB	fast atom bombardment
h	hour
HPLC	high-performance liquid chromatography
Hz	hertz
J	coupling constant (NMR spectra)
LAH	lithium aluminium hydride
m	multiplet (NMR spectra)
M	moles per litre
MHz	megahertz
min	minutes
mmol	millimole(s)
mp	melting point
MS	mass spectrometry
m/z	mass to charge ratio (mass spectrometry)
NMR	nuclear magnetic resonance
ppm	parts per million (NMR spectra)
^{i}PrOH	*i*-propanol
q	quartet (NMR spectra)
R_f	retention factor (chromatography)
RP-HPLC	reverse phase high-performance liquid chromatography
RT	room temperature
s	singlet (NMR spectra)
t	triplet (NMR spectra)
TEA	triethylamine
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
p-TSA	*p*-toluenesulphonic acid
UV	ultra-violet

NOMENCLATURE AND NUMBERING SYSTEM



The opioid compounds prepared in this thesis are named using 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-dihydroxyindolo[2',3':6,7] morphinan as a base.

GLOSSARY

Agonist – a substance that interacts with a receptor, causing a physiological response.

Antagonist – a substance that blocks the action of an agonist by binding to the receptor.

Affinity – the binding power of a compound.

Efficacy – the degree of activation of the receptor.

Pharmacophore – the core structure of a pharmacologically active series of compounds

Salt bridge – electrostatic interaction formed between a negatively charged species and a positively charged species

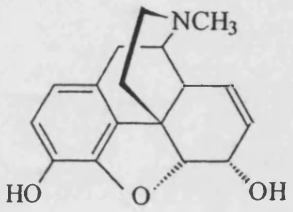
Hydrogen Bonds – dipole-dipole interactions between a non-bonding electron pair of a heteroatom (donor) and an electron deficient hydrogen atom (acceptor).

Hydrophobic Bonds – occur when non-polar sections of molecules are forced together by a lack of water solubility thus excluding water molecules and lowering the energy of the system.

1. INTRODUCTION

1.1 BACKGROUND

The analgesic properties of morphine (**1**) have been exploited since time immemorial. The adverse effects on the respiratory system and gastrointestinal tract, along with the addictive potential of the drug are, however, highly undesirable.[1] Morphine was first isolated from the poppy, *Papaver somniferum*, in 1803 by the German pharmacist Serturner. The chemical structure was however only determined in the 1920's by Gulland and Robinson,[2] with the total synthesis following in 1952.[3]



1

Opioids such as morphine are known to interact with receptors in the central nervous system and periphery, causing analgesia.[1] The existence of distinct types of opioid receptors was first proposed by Martin in 1967.[4] Since then, a large amount of evidence supporting this theory has been reported[5] and at least three receptor types (μ, δ and κ) have now been sequenced and cloned.[6] Each of these three opioid receptors is coupled to a G-protein and consists of an extracellular N-terminal, seven transmembrane domains and an intracellular C-terminal portion (**Fig. 1**)[6]. The peptide sequence of the intracellular and transmembrane portions of the three receptor types is highly conserved,[7] whereas the extracellular region varies significantly from one receptor type to another.

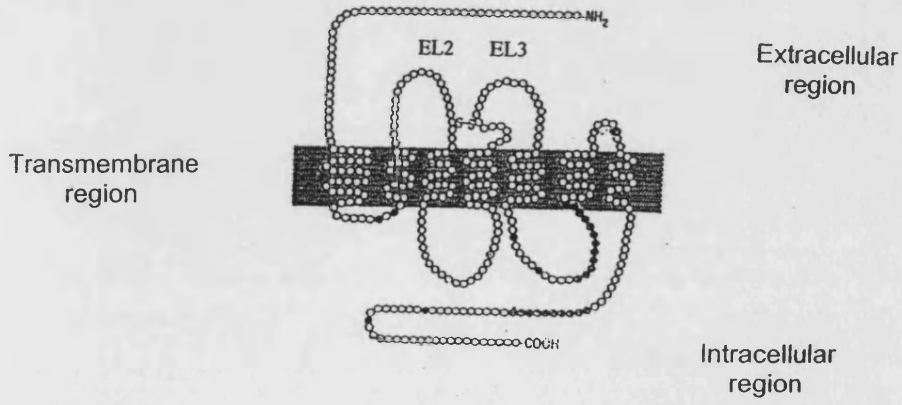


Fig. 1 The seven transmembrane domains of an opioid receptor.

Although each of the three receptors is able to mediate analgesia, additional pharmacological responses exist which are specific for the receptor types (**Table 1.**)

μ	κ	δ
Analgesia	Analgesia	Analgesia
Respiratory depression	Diuresis	Convulsions
Constipation	Sedation	
Euphoria	Dysphoria	
Dependence		

Table 1. Pharmacological effects mediated by different opioid receptor types.

1.2 ENDOGENOUS LIGANDS

Peptidic substances displaying morphine like properties were isolated from brain tissue by various groups in the mid 1970's.[8] The active material was termed *enkephalin* by Hughes, *et al.*[9] Enkephalin isolated from pig brain was found to consist of two pentapeptides, Tyr-Gly-Gly-Phe-Met(OH) and Tyr-Gly-Gly-Phe-Leu(OH) in a ratio of approximately 4:1. These two peptides differ only in their C-terminal amino acid and are generally referred to as methionine-enkephalin (*Met*-enkephalin) and leucine-enkephalin (*Leu*-enkephalin).

In functional assays, these two enkephalins display good efficacy, with *Met*-enkephalin showing greater activity than morphine. *In vivo*, however, the endogenous ligands are less potent than morphine and have a shorter duration of action.[10] This is due to the rapid deactivation of enkephalins by enzymatic cleavage of the Tyr-Gly and Gly-Phe bonds. Enzymes that preferentially act on enkephalins are localised in the vicinity of opioid receptors,[11] possibly exerting physiological control over endogenous opioid peptide activity.

It was hoped that the discovery of endogenous peptide ligands would lead to a non-addicting analgesic, however, dependence upon these compounds has been shown.[12]

Various *Met*- and *Leu*-enkephalin based peptides displaying opioid activity have been isolated from the adrenal medulla. The pituitary gland has also yielded various opioid peptides, many of which are derived from β-lipotropin, a relatively large peptide which itself displays no opioid properties.[14] It is notable that of all the β-lipotropin fragments, only those containing amino acids 61-65 (analogous to *Met*-enkephalin) show opioid like effects.[15] Dynorphin, a peptide derived from prodynorphin, has also been isolated from the pituitary gland.[16]

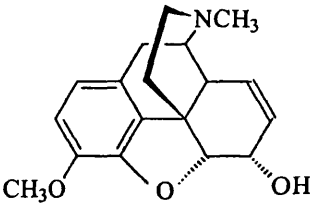
Although endogenous peptide ligands are usually non-selective, certain inherent selectivities have been noted.[17] β-Endorphin interacts mainly with μ- and δ- receptors. *Leu-* and *Met-*enkephalin preferentially act on δ-receptors, while dynorphin displays a certain degree of κ-selectivity.

Synthetically, exchanging one or more of the naturally occurring (L)-amino acids for the less common (D)-isomer produces peptide ligands with greater resistance to enzymatic cleavage. Exchanging one of the amino acids in the sequence for an alternative amino acid may also increase this resistance[13] (see compounds **12-14**, section 1.3). A good example of this is the synthetic peptide ligand, DAMGO (**12**, section 1.3), in which Gly^2 of enkephalin has been exchanged for $D\text{-}Ala^2$, Phe^4 has been exchanged for $MePhe^4$ and $Gly\text{-}ol^5$ replaces the terminal Met^5 or Leu^5 residue.

1.3 AGONISTS

Opioid agonists elicit analgesia by acting within the central nervous system. The primary areas of action are those concerned with the perception of pain and respiratory control. In contrast to anaesthetics therefore, opioid analgesics cause no loss of consciousness.

Although clinically the most beneficial property of morphine is its ability to relieve severe pain, undesirable side effects such as respiratory depression, altered mood, physical dependence, constipation and vomiting are experienced.[18,19] The naturally occurring opioid codeine (**2**), which has lower potency but is also less addictive,[1] is found in many commercially available pharmaceutical products. Heroin (**3**), an acylated derivative of morphine, was synthesized in an attempt to find a compound possessing the analgesic properties of morphine without the undesirable side effects. Although heroin is a marginally better analgesic than morphine, it has a substantially greater liability to abuse.



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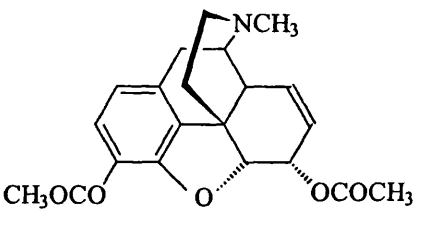
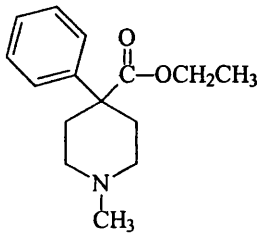


3

The search for analgesics devoid of addictive properties lead to the development of various totally synthetic compounds, originally focussing on four main classes,[19] namely; 4-phenylpiperidines [*eg.* pethidine (**4**)], diphenylpropylamines [*eg.* methadone (**5**)], morphinans

[*eg.* levorphanol (**6**)], and 6,7-benzomorphans [*eg.* metazocine (**7**)]. The majority of *N*-methyl compounds of the above classes display agonist nature.



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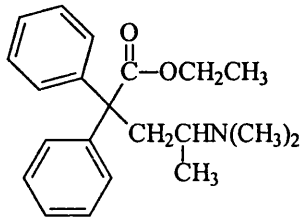

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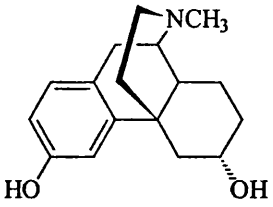

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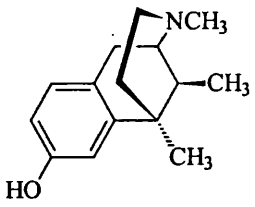

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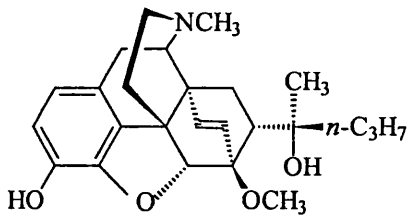
Two semi-synthetic compounds from the orvinol series, etorphine (**8**) and buprenorphine (**9**), have found wide applicability in animal[20] and human[21] use respectively. While etorphine displays high agonist potency, particularly in animals, buprenorphine is a μ-partial agonist/κ,δ-antagonist. The key step in the synthesis of these compounds is a Diels-Alder reaction between methyl vinyl ketone and the naturally occuring epoxymorphinan, thebaine (**10**).



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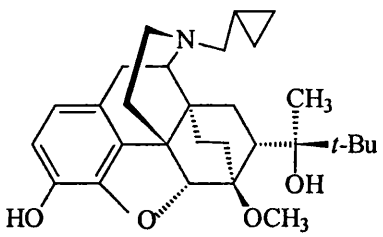

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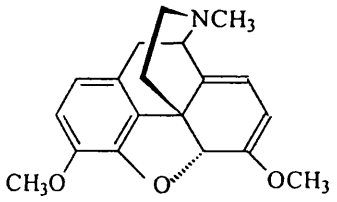

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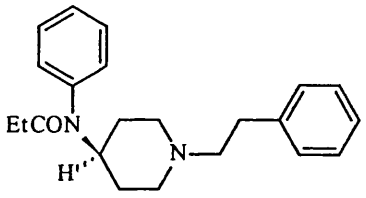
Since the discovery of endogenous ligands and distinct receptor types, a challenge has been to synthesise not only compounds which show selectivity due to relative efficacy (eg. buprenorphine, **9**), but also agonists that show μ-, δ- and κ-selectivity in binding. Such ligands would allow the pharmacological roles of the individual receptors to be determined. The synthesis of selective radiolabelled ligands would provide further insight into receptor-ligand interactions.

The original μ-agonist, against which other compounds were measured, was morphine. Although morphine did not show selectivity in binding assays, μ effects dominated *in vivo* assays. In the 1960's, fentanyl (**11**)[22], a μ-agonist showing short duration of action and almost 200 times the activity of morphine, went into clinical use.



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The endogenous peptide ligands, endomorphin-1 and -2 also show μ-selectivity.[17] The μ-selective agonist currently used in binding and *in vitro* functional assays is DAMGO[7,23] (**12**, D-Ala-MePhe-Gly-ol enkephalin).

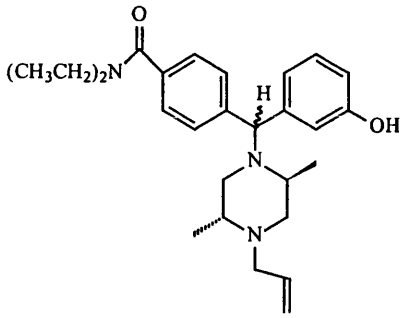
H-Tyr^1-D-Ala^2-Gly^3-MePhe^4-Gly^5-OH **12**

H-Tyr^1-D-Ala^2-Gly^3-Phe^4-D-Leu^5-OH **13**

H-Tyr^1-D-Pen^2-Gly^3-Phe^4-D-Pen^5-OH **14**

Most δ-selective agonists are derived from the endogenous peptide ligands *Met-* and *Leu-*enkephalin, which act predominantly with δ-receptors. Incorporation of unnatural D-amino acids in the peptide sequence offers greater resistance to enzymatic hydrolysis. DADLE (**13**, D-Ala-

D-Leu enkephalin), and more recently DPDPE (**14**, D-Pen-D-Pen enkephalin) have been used as standard δ-selective agonists in binding assays.[24] It was only in the 1990's that (±)BW 373 U86 (**15**), the first non-peptide δ-agonist was discovered.[25] The racemic compound has since been resolved and modified to give the methyl ether, (+)SNC 80 (**16**),[26] which displays high affinity and greater selectivity than either (+) or (-)BW 373 U86.



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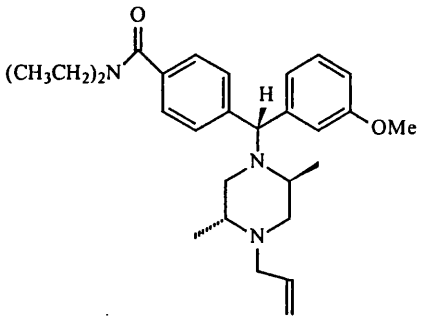

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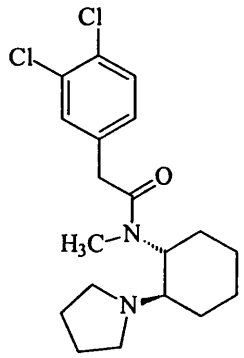
The first κ-selective agonist to be developed was the non-peptidic, arylacetamide ligand U50488 (**17**).[27] Subsequent modification of this compound has lead to other κ-selective agonists, including U69 593 (**18**) and C1 977 (**19**).[7] Although most κ-selective agonists belong to the arylacetamide class, agonist properties have also been reported for various bicyclononanone[28] (eg. HZ2, **20**) and benzomorphan[29] derivatives (eg. CCB, **21**).



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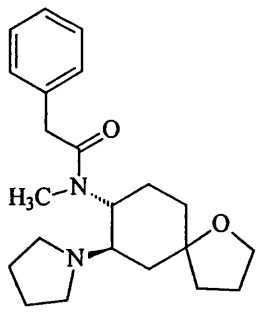

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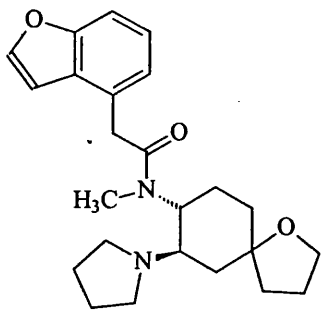

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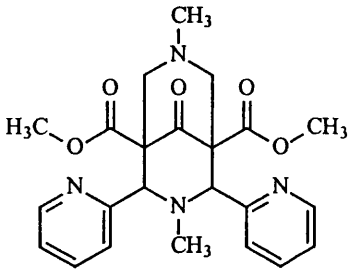

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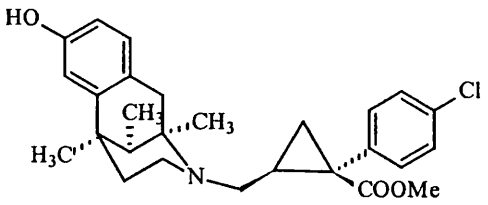

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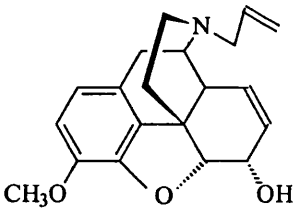
1.4 ANTAGONISTS

In the early 1940's it was discovered that the effects of morphine in dogs (drowsiness, uncoordination and vomiting), could be reversed by treatment with *N*-allylnorcodeine (**22**) or *N*-allylnormorphine (**23**, nalorphine).[30] By administering nalorphine prior to morphine, the respiratory depression and analgesic effects usually induced by morphine could not be detected. Nalorphine was hence antagonising the effects of morphine and could therefore be used in the treatment of morphine overdose and resultant respiratory depression. Reports later emerged, however, in which nalorphine displayed analgesic properties,[31] which were later attributed to an agonist response at the κ-opioid receptor.



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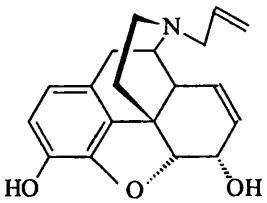

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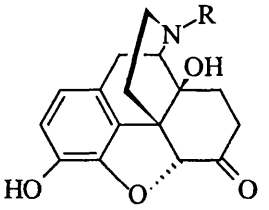
A compound that displays antagonist properties will cause a shift to the right in the dose-response curve of a standard agonist. If compounds are competitive antagonists, interacting reversibly with the receptor, a parallel shift will be seen. Selective opioid antagonists are primarily used as pharmacological tools to gain a better understanding of the interaction of endogenous opioid peptides and newly discovered agonists with the various receptor types.

The first pure opioid antagonist to be discovered was naloxone (**24**). Apart from its usefulness as a pharmacological tool, naloxone is now used in the treatment of opioid induced respiratory depression as well as CNS injuries, and the regulation of blood pressure in various forms of shock.[32] Naltrexone (**25**), the *N*-cyclopropylmethyl analogue of (**24**) is a pure opioid antagonist

which displays higher oral activity and longer duration of action than (**24**).[33] Naltrexone has proved useful in the treatment of opioid dependence and alcoholism.



24 R = allyl

25 R = cyclopropylmethyl

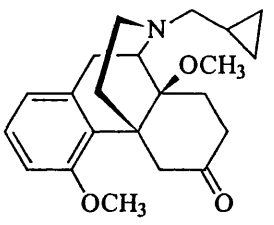
Both naloxone (**24**) and naltrexone (**25**) are competitive antagonists at μ-, κ- and δ-receptors, although μ-selectivity has been shown at low dose concentrations *in vivo*.

If the compounds are non-competitive antagonists and form an irreversible interaction/bond with the receptor, the dose-response curve will be shifted to the right and down (*ie.* a lower maximum response will be achieved.)

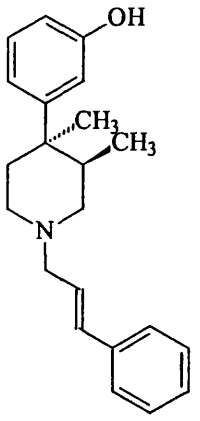
Irreversible antagonists are useful in the assessment of agonist efficacy since they immobilize the receptor reserve in a preparation, hence increasing the efficacy demand on ligands to produce a response.[34] The apparent efficacy of the ligand is therefore reduced and less than maximal response is observed. The efficacy of the ligand is directly related to the concentration of irreversible antagonist required to produce such an effect. Selective non-competitive ligands have proved particularly valuable since they permit complete knockout of one particular receptor, allowing the role of each of the individual receptor types to be more easily determined.

1.4.1 μ-SELECTIVE ANTAGONISTS

The first non-peptidic, competitive, pure opioid antagonist to show μ-selectivity *in vitro* and *in vivo*, was cyprodime (**26**).[35] Although (**26**) displays a high degree of μ-selectivity, it shows lower μ-affinity and lower antagonist potency than naloxone (**24**).

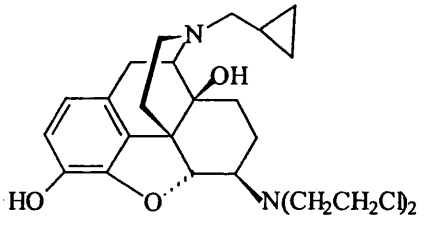


In the late 1970's it was shown that *N*-substituted 4-phenylpiperidines displayed non-selective antagonist activity at opioid receptors.[36] It was not, however, until much later that μ-selectivity was shown for a compound of this type. Since *N*-substitution did not influence the antagonist nature of the compounds, various changes were made to this region of the molecule, in the hope of increasing μ-selectivity. The most selective compound thus far, which employs a cinnamyl group as the N-substituent, has been disclosed by Thomas *et. al.* (**27**).[37]



27

β-CNA (**28**), a non-competitive opioid antagonist, binds irreversibly to all three opioid receptor types via the alkylating function at C-6.[38] β-FNA (**29**) is a μ-selective, irreversible opioid antagonist,[39] based on the structure of β-CNA. It was thought that replacing the nitrogen mustard with a less reactive Michael acceptor, such as a fumarate group, would confer greater selectivity during covalent bond formation. Although β-FNA showed good selectivity during both *in vivo* and *in vitro* assays, agonist properties were initially displayed, after which the antagonist properties became dominant.[39,40] The agonist properties were attributed to an interaction at the κ-opioid receptor.



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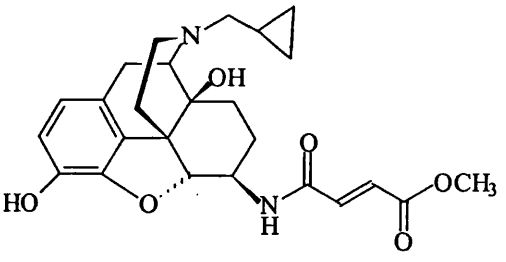
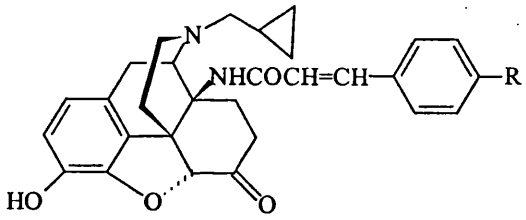


29

Lewis *et al.*, developed the compound C-CAM (**30**), a μ-selective antagonist showing long duration of action.[41] This compound is particularly noteworthy since it displays only antagonist properties without initial agonist effects.[42] Initially, it was thought that the cinnamoylamino substituent served as a Michael acceptor for nucleophilic addition.[43] However, it has since been shown that no covalent bond is formed and the interaction is hence pseudo-irreversible.[44] The observed binding can be explained in terms of a strong lipophilic interaction. The apparent anomaly which exists, in that C-CAM is highly μ-selective in behavioural assays but only moderately selective in binding assays, can be explained by C-CAM binding reversibly to all three receptor types, while only the interaction with μ-receptors is wash-resistant.[44] This is an example of selectivity amplification. M-CAM (**31**),[45] the methyl analogue of C-CAM has since been reported to show improved selectivity over C-CAM, while retaining pure antagonist properties.



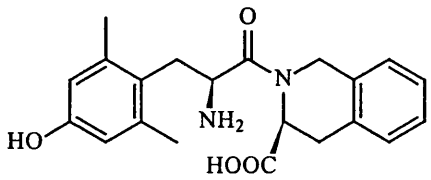
30 (R = Cl)

31 (R = CH_3)

Following on from the work on C-CAM, Derrick *et al.* have now reported 3-deoxy C-CAM.[46] This compound is noteworthy in that it only displays reversible high affinity, μ-antagonism. Why the removal of a phenolic substituent should result in a loss of irreversibility, but not affinity or selectivity, however, remains to be determined.

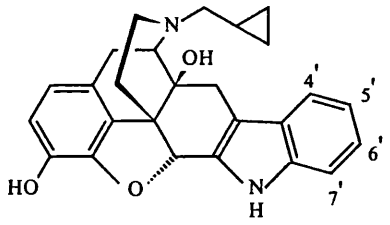
1.4.2 δ-SELECTIVE ANTAGONISTS

Reasonable success has been achieved in obtaining δ-selective competitive opioid antagonists through the modification of endogenous peptide ligands.[47] Two peptide based δ-antagonists which show good selectivity are H-Tyr-Tic-Phe-Phe-OH (TIPP) and H-Tyr-Tic-Phe-OH (TIP),[48] where Tic represents a 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid residue. Currently, the dipeptide DMT-Tic (**32**) shows the highest selectivity and affinity of the δ-selective peptide antagonists,[49] and has been reported to be active after both central and peripheral administration.



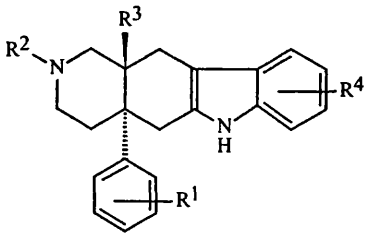
32

The first δ-selective non-peptide competitive antagonist was naltrindole (**33**). This compound was designed by Portoghese *et al.*,[50] and was based on the "message-address" concept, in which it was postulated that there are two recognition subsites. Interaction at the "message" subsite relates to opioid activity, while interaction at the "address" subsite controls selectivity.[51] The naltrexone pharmacophore provided the message portion of the molecule (opioid antagonist activity), while the indolic phenyl group, mimicking a key element in Phe[4] of Leu-enkephalin, conferred δ-selectivity.[52] The address portion of the molecule was held in the correct orientation with respect to the message portion by the pyrrole spacer. Administration of naltrindole decreases physical dependence in patients who receive morphine regularly. Naltrindole was also found to exhibit immunosuppressant properties in rat models, without the cytotoxic effects experienced with cyclosporin.[53] Several ligands closely related to naltrindole have since been synthesized and display good δ-selectivity. Substitution at the 7'-position, for example with a bromine, hydroxyl, or benzyloxy-group has been particularly well tolerated; either retaining or increasing selectivity.[54]



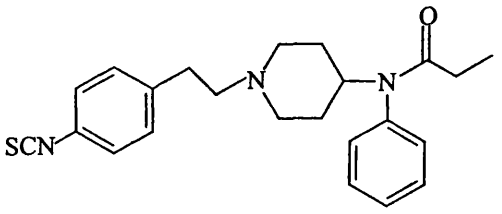
33

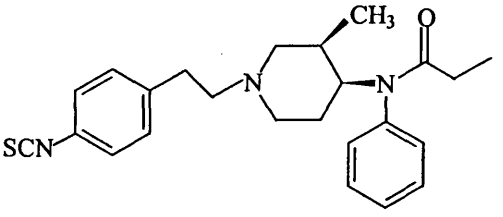
More recently, δ-selective compounds, incorporating only the essential portions of the naltrindole structure, have been prepared (**34**).[55] These indolo- and benzofurooctahydroisoquinoline compounds are generally more selective but less potent than naltrindole.



34

FIT (**35**)[56] and SUPERFIT (**36**)[57] were two of the earliest δ-selective irreversible antagonists discovered. Both of these ligands however displayed partial agonist properties during *in vivo* assays.

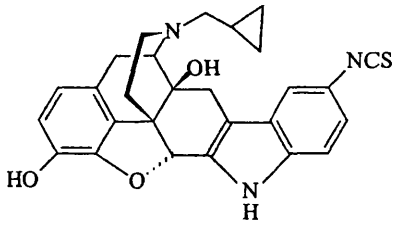




35 **36**

By designing an irreversible antagonist based on the structure of the known reversible antagonist, naltrindole (**33**), Portoghese *et al.* were able to report the compound NTII (**37**),[58] which showed δ-selective irreversible antagonism in the absence of initial agonism. A more recently disclosed δ-selective irreversible antagonist, BNTII (**38**),[59] is similarly based on the N-benzylnaltrindole (BNTI) structure with an isothiocyanate group again providing the means for an irreversible interaction.

Interestingly, BNTI (**39**)[59,60] shows a similar profile to BNTII (**38**), although there exists no means for covalent bond formation. At present this is explained by a strong lipophilic interaction between ligand and receptor, resulting in binding that is extremely tight, albeit reversible.



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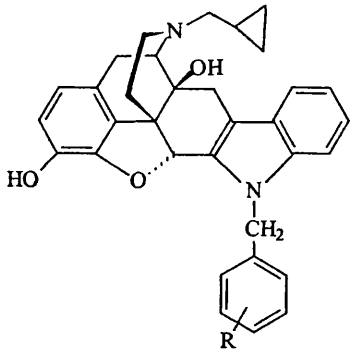
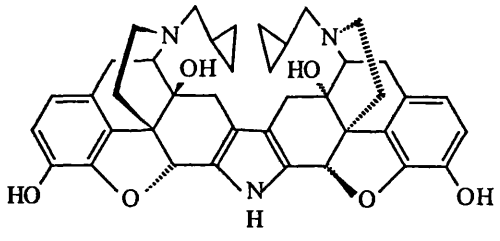


38 (R = NCS)
39 (R = H)

1.4.3 κ-SELECTIVE ANTAGONISTS

Until very recently, attempts to synthesize a peptidic κ-selective, reversible antagonist based on the structure of the κ-selective endogenous peptide ligand, dynorphin A, have been unsuccessful.[17] Schiller *et al.*, have now reported dynantin, the first highly potent dynorphin A derived antagonist showing good κ-selectivity.[61]

The prototype κ-selective antagonist, norbinaltorphimine (**40**, norBNI), is a non-peptidic, bivalent ligand showing a high degree of selectivity both *in vitro* and *in vivo*.[62] There is however a delay between the administration of norBNI and the peak κ-selective antagonist effect.[63] Poor solubility and extremely long duration of action further complicate the pharmacological profile of this drug. Literature reports show the effects of norBNI lasting up to 7 weeks in mice and 11 weeks in pigeons.[64]

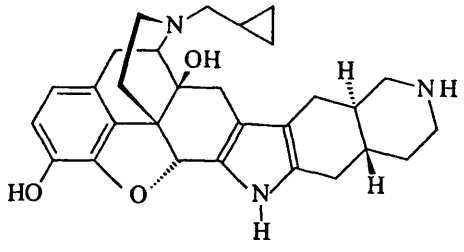


40

The long duration of action could be explained in terms of an irreversible interaction with the opioid receptor, however the fact that effects of norBNI are surmountable,[65] suggests this is not

the case. A more plausible explanation would be that the large size of the bimorphinan structure impedes the diffusion of the drug through the blood-brain barrier, causing slow onset of action. Poor elimination of the drug from the brain would account for the long duration of action.

Studies have shown that the second pharmacophore is not essential, but rather, it is the presence of the second basic nitrogen which is crucial in retaining good κ-antagonist selectivity.[66] It has been postulated that this basic nitrogen mimics the guanidine moiety of the basic Arg^7 residue of the endogenous, κ-selective peptide, dynorphin.[51] At the same time, the basic nitrogen group decreases δ-antagonist potency.[67] With the recent cloning of opioid receptors, it has become possible to examine these postulates by means of site-directed mutagenesis and chimera studies. Using various μ/κ chimeras, κ-selectivity for morphinan-type antagonists was shown to reside in extracellular loop (EL) 3 of the κ-opioid receptor.[68] Glu 297, an acidic residue in EL 3 which could interact with a basic "address" moiety of the morphinan ligand was identified,[69] and its role in conferring κ-selectivity was further confirmed by site-directed mutagenesis studies.[69,70] This acidic residue is unique to the κ-receptor, the analogous position being occupied by a bulky Trp (Trp 284) and basic Lys (Lys 303) residue in the δ- and μ-receptors, respectively.



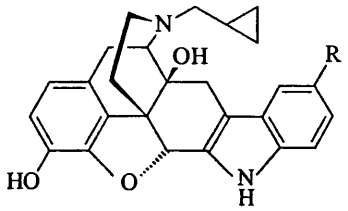
41

In an effort to improve the *in vivo* properties of norBNI, Portoghese *et al.* synthesised the piperidine (**41**),[66] which has a basic nitrogen held by a pyrrole spacer in a position similar to that of the second basic nitrogen in norBNI, but which lacks much of the second bulky morphinan group. *In vitro*, (**41**) showed slightly better selectivity than nor-BNI, however the 2% overall yield prevented *in vivo* evaluation. A readily accessible method for preparing this, or closely related compounds would be highly desirable. Furthermore, derivatives of the secondary amine could be prepared and their antagonist properties evaluated.

The interatomic distance and spatial arrangement of the two basic centres is crucial in conferring κ-selectivity in morphinan-type antagonists. This distance, as measured for norBNI, is approximately 11 Å.[71] Additionally, it has been found that substituting naltrindole (**33**) with a basic group at the 5' position decreases δ-affinity.[67] With this in mind, compounds with an

amidine[67] (**42**), amide-amine[72] (**43-44**) or amidine-amine group[72] (**45**) attached to the 5'-position of naltrindole were synthesised, showing good κ-selectivity.

Results for the amidines indicated that selectivity increased as the length of the side chain increased, however, the chain length was not extended beyond the most selective compound (n=1, m=3). Jales *et al.*[73] then went on to report the most selective amidine compound thus far (**42**, n=2, m=3), as well as a series of 5'-amido-substituted compounds[74] (**46**) which showed modest selectivity, despite the lack of a second basic centre.



42 R = $CH_nNHC(=NH)(CH_2)_mCH_3$ (n=1,2 m =1,2,3,4)

43 R = $CONHCH_2CH_2N(CH_nCH_3)_2$ (n=0,1)

44 R = $CH_2NHCO(CH_2)_3N(CH_3)_2$

45 R = $CH_2NHC(=NH)(CH_2)_nN(CH_3)_2$ (n=1,3)

46 R = $CH_2CH_2NHCOCH_nCH_3$ (n=1,2,3)

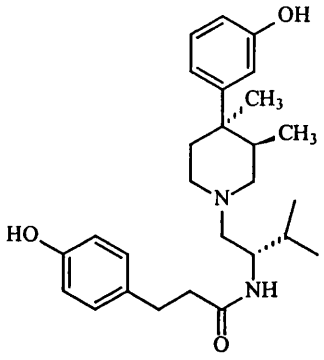
47 R = $NHC(=NH)NH_2$

Substitution of the 5'-position of naltrindole with a basic guanidine group gave the highly κ-selective anatgonist GNTI (**47**).[75] It is noteworthy that a δ-selective compound has been changed into a κ-selective compound, simply by the addition of a basic group. Interestingly, Sharma *et al.*,[76] have shown that moving the guanidine substituent to the 6'-position gives a selective κ-agonist. With the guanidine group in the 7'-position, the compound becomes a selective δ-antagonist. This is consistent with previous findings that substitution at the 7'-position of naltrindole does not affect δ-selectivity (**section 1.4.2**).

Portoghese recently published[77] a comprehensive range of 5'-substituted indolo-morphinan ligands, allowing investigation into the optimum distance between the second basic centre and the core naltrindole structure, as well as the effect of various substituents. Guanidine, amidine, quaternary ammonium and amine side chains all displayed κ-antagonism with potency decreasing in this order. He concluded that κ-antagonist efficacy appeared to be related to both the pKa of the side chain and the distance between the two cationic centres. Thus far, GNTI (**47**) is the most potent and selective κ-antagonist to have been reported. Preliminary data reported at the 2001 Annual Scientific Meeting of the College on Problems of Drug Abuse

(CPDD),[78] would indicate that the *in vivo* effects of GNTI are long lasting; a situation similar to that found for norBNI.

The phenyl piperidines, an unrelated class of compounds which have previously been associated with μ-antagonists, have also provided selective κ-antagonists such as (**48**)[23] and (**49**).[79]



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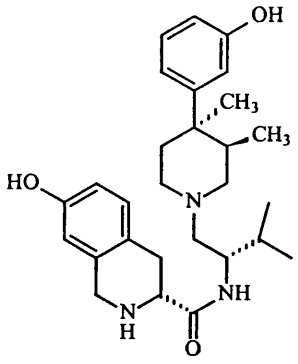
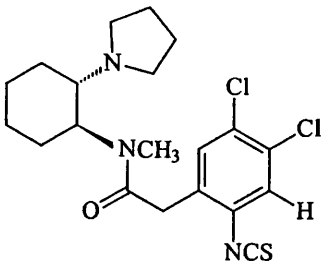


49

UPHIT (**50**), one of the first κ-selective irreversible antagonists was disclosed by Rice *et. al.*[80] The compound was designed by the addition of an acylating isothiocyanate group into the structure of the known κ-agonist U50488 (**17**). A more recently reported compound, DIPPA (**51**),[81] which also makes use of an isothiocyanate acylating group, is based on the finding that ICI 199411 (**52**) shows potent κ-selective agonism.



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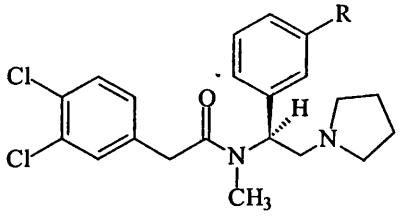


51 (R = NCS)

52 (R = H)

Both UPHIT (**50**) and DIPPA (**51**) display short term agonism, followed by long term antagonism. This can be seen as a consequence of their design, since both compounds are based on the structures of known agonists. This problem has restricted the use of these two ligands in investigating κ-receptor function.

Similar problems were encountered in the early irreversible antagonists selective for the δ-opioid receptor. This problem however, was overcome by designing non-competitive ligands based on competitive δ-antagonist structures. A possible reason why this was not exploited in the design of early irreversible κ-antagonists is the limited opportunities in the structure of the original κ-selective antagonist, norBNI (**40**), for the introduction of an electrophilic group.

The only viable position is the pyrrole N-atom, which could be substituted with a benzyl group (into which an electrophile could be introduced), in a manner analogous to that used in the design of BNTI (**39**) and BNTII (**38**), irreversible δ-antagonists based on the reversible δ-antagonist naltrindole.

More recently, however, 5'-substituted naltrindole derivatives, and particularly guanidinium compounds, have shown substantially greater selectivity than that of norBNI. Basing the design of an irreversible κ-antagonist on these structures would therefore seem more favourable. This could be done either by introducing a benzyl group at the indolic nitrogen (again with the possibility of substituting with an electrophile such as isothiocyanate) or by introducing an electrophilic group into the 5'-basic side chain.

As mentioned previously, irreversible antagonists can also be used in the assessment of agonist efficacy. While β-FNA (**29**) and C-CAM (**30**) have been used both *in vivo* and *in vitro* to assess μ-efficacy, no such procedures have been undertaken with the available κ-irreversible ligands, UPHIT (**50**) and DIPPA (**51**). A κ-selective irreversible ligand, solely displaying antagonism would prove an extremely valuable tool in assessing the relative efficacy of standard agonists, both those displaying selectivity for the κ-receptor, as well as those with affinity for more than one opioid receptor type.

1.5 THE SIGNIFICANCE OF THE κ-OPIOID RECEPTOR

Originally, research into κ-selective opioid ligands focussed on potential analgesics.[82] Since κ-agonists do not cause euphoria, it was hoped that this class of compounds could provide a potent, non-abusable analgesic. The dysphoric side effects, however, have precluded the use of pure κ-agonists in the treatment of pain.

Although the analgesic properties of opioids have long been recognised, it has become increasingly apparent that they have a significant role to play in a wider range of clinical situations.

Cocaine administration has been shown to up-regulate κ-opioid receptors, causing an increase in the levels of both dynorphin and dynorphin mRNA.[83] It is therefore conceivable that κ-opioid

systems play a role in modulating the effects of cocaine. Recently there has been significant interest in the role of κ-opioid agonists in cocaine abuse. In rodents, κ-opioid agonists were found to block cocaine-induced place preferences and hyperactivity.[84] Acute administration of the κ-agonists, U50 488 and spiradoline, has also been reported to reduce self-administration of an intermediate dose of cocaine in rats.[85] Additionally, the κ-antagonist norBNI, potentiates the discriminative stimulus effects of cocaine.[86]

In post-detoxification treatment of heroin addicts, Rothman *et al.*[87] have shown that a combination of buprenorphine (μ-partial agonist/κ-antagonist) and naltrexone (μ-antagonist) produced a greater positive response to treatment than with naltrexone alone, indicating a possible role for κ-antagonists in the treatment of drug addicts. It is still unclear, however, if the presence of a μ-partial agonist contributes to the positive response. This can be supported by the fact that in rat models, administration of norBNI has no effect on morphine or heroin self-administration.[88]

The use of κ-agonists as alternatives to hyperosmotic agents in the treatment of cerebral edema of the focal ischemia type, has also been postulated.[89]

Newman *et al.*[90] have suggested a role for κ-antagonists in determining the underlying mechanisms that cause the motor fluctuations that develop during treatment of Parkinson's disease, while norBNI (**1**) has been reported to improve recovery after traumatic brain injury in rat models.[91] During feeding studies using rats, administration of norBNI has been shown to reduce deprivation intake and suppress other forms of food intake. The κ-receptor has also been implicated in polydipsia (alcohol dependence), since norBNI was able to decrease drinking in genetically polydipsic mice.[92]

1.6 SPECIFIC AIMS

While norBNI (**40**) has proved a useful ligand for the study of κ-receptors, there is still significant interest in the development of alternative ligands, with, for example improved pharmacokinetic properties. It was believed that less lipophilic, lower molecular weight ligands might display a more favourable pharmacological profile.

In order to extend the range of ligands available, it was decided to synthesise further series of 5'-amido, amidino, imidazoline and urea substituted compounds, and to submit these compounds for pharmacological evaluation. In this way, it would be possible to further probe the effects of varying the distance between the two basic centres. Since urea derivatives are less basic than amidine derivatives, and amido derivatives are non-basic, it would also be possible to investigate the level of basicity required for κ-selectivity. The most highly κ-

selective compound reported thus far is the 5'-guanidino-substituted, GNTI (**47**). It was envisaged that further substitution of the guanidine functionality might reveal compounds with increased κ-selectivity and/or affinity and allow the introduction of new functional groups. In this regard, a terminal amine group was particularly desirable, since this can readily be converted to an isothiocyanato group which could confer irreversible properties to these ligands.

It was hoped that molecular modelling studies would allow us to investigate the interactions between the synthesised ligands and the κ-receptor. In addition, selectivity could be investigated by docking the various ligands into all three opioid receptors. This would allow us to design new, potentially selective ligands for the κ-opioid receptor.

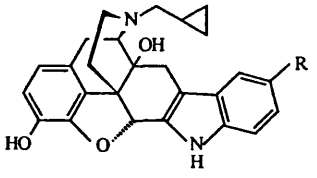
2. SYNTHESIS

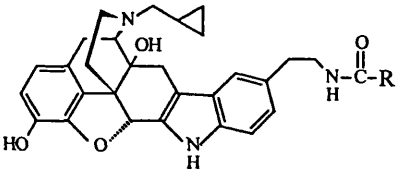
2.1 AMIDE SUBSTITUTED LIGANDS

2.1.1 DESIGN RATIONALE

In a 1995 patent[72] Portoghese and Olmsted disclosed a series of aminoamide compounds (**53-55**), showing selectivity for the κ-opioid receptor. Although the amide group is not a basic functionality, due to delocalisation of the lone pairs across the carbonyl, selectivity was demonstrated for these compounds. This could however be attributed to the basicity of the distal amine in the side chain. At first glance this amine group would not appear to be in a suitable position for interaction with the receptor. It is feasible however, that the alkyl chain could fold back upon itself, thereby placing the nitrogen in close proximity to the acidic Glu 297 residue.

Within our group,[74] a series of amide derivatives (**56-58**) have also been prepared. These compounds again show a degree of κ-selectivity – albeit lower than that reported for the aminoamides (**53-55**). In this case, however, there is no basic group in the side chain.





53 R = $CONH(CH_2)_2N(CH_3)_2$

54 R = $CONH(CH_2)_2N(CH_2CH_3)_2$

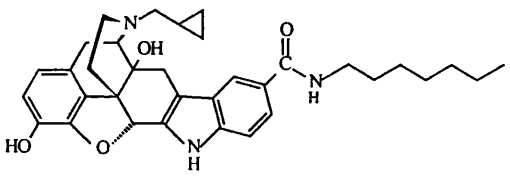
55 R = $CH_2NHCO(CH_2)_3N(CH_3)_2$

56 R = CH_2CH_3

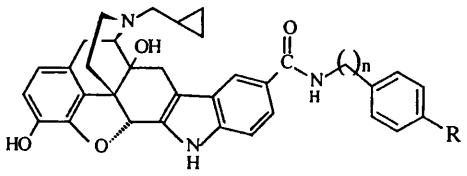
57 R = $(CH_2)_2CH_3$

58 R = $(CH_2)_3CH_3$

For ligands (**56-58**), an increase in the acyl chain length was accompanied by an increase in κ-selectivity, seemingly confirming our belief that lipophilic interactions are also important in the κ-address. If hydrophobic interactions are important, an obvious extension of this work would be to synthesise a series of amides with a longer alkyl group (**59**) or an aryl ring in the side chain (**60-63**). The resultant increase in lipophilicity and potential for strong hydrophobic interactions could further increase κ-selectivity. Whereas for compounds (**53-54**), the amide functionality is attached directly to the naltrindole core, for compound (**55**) there is a methylene spacer before the amide group. Since receptor binding data for compounds (**53–55**) were highly similar,[72] it was decided to attach the amide group directly to the 5'-position of naltrindole as in compounds (**53-54**).



59

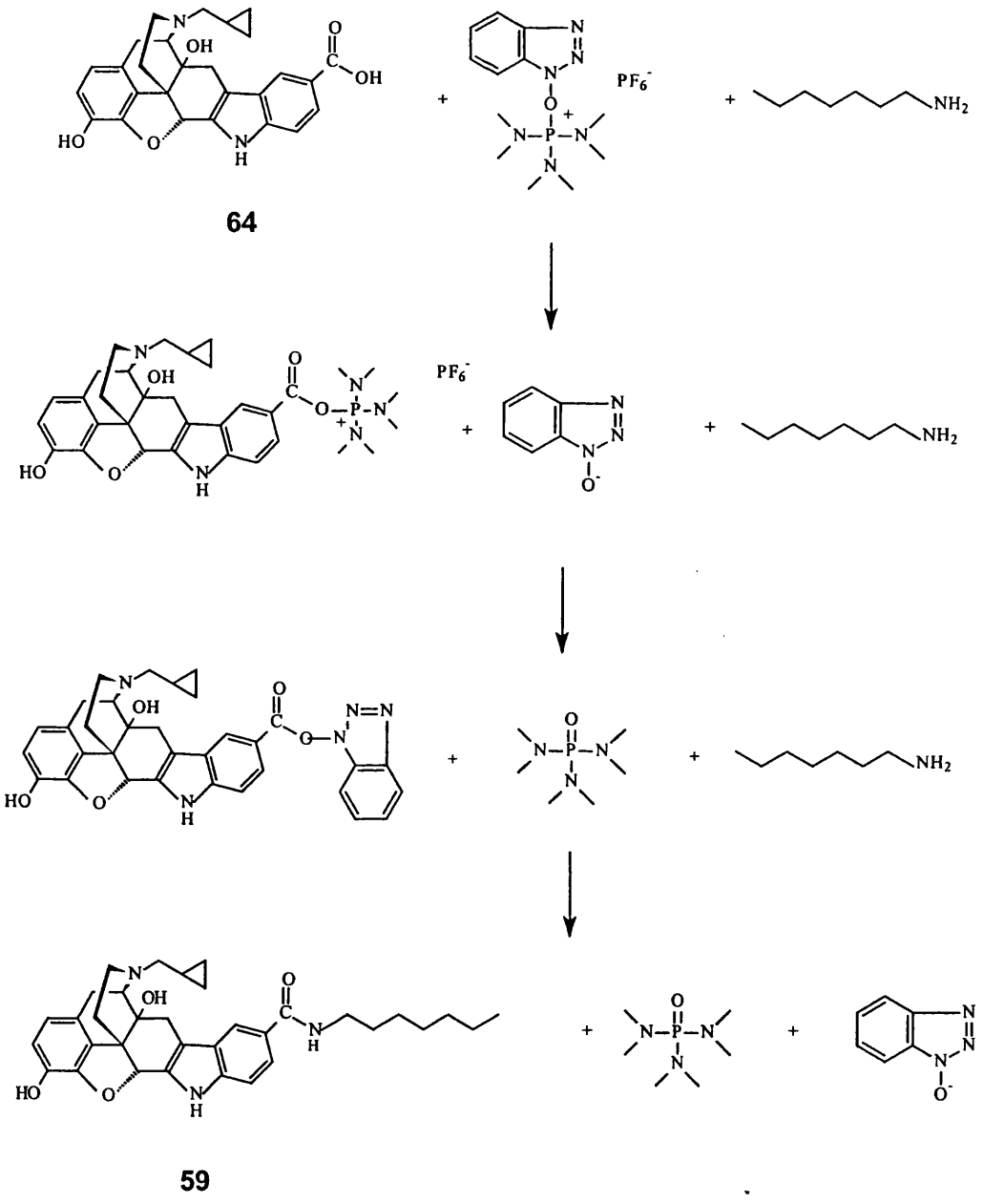


60 n = 1, R = H
61 n = 2, R = H
62 n = 4, R = H
63 n = 1, R = OMe

2.1.2 SYNTHESIS

Amides are commonly formed *via* the acylation of amines, by activated carboxylic acid derivatives, such as acyl halides,[93] anhydrides,[94] or carboxylic esters.[95] Perhaps the most widely used method is the reaction of a carboxylic acid with a coupling agent (eg. DCC), followed by displacement with an amine.[96] Carbodiimide derivatives are most commonly used for this purpose, although the reaction may also be promoted by reagents such as $ArB(OH)_2$[97] and $POCl_3$.[98]

Compounds (**53-54**) were synthesised *via* the benzotriazol-1-yloxytris(dimethylamino)-phosphonium hexafluorophosphate (BOP)[99] assisted coupling of (**64**) with the required amine.[72] Accordingly, we accomplished the synthesis of (**59**) using (**64**), BOP and heptylamine in the presence of triethylamine (**scheme 1**). The reaction proceeded well, giving 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N-heptyl)amido-3,14-dihydroxyindolo[2',3':6,7]-morphinan (**59**) in a yield of 83%. The carboxylic acid precursor (**64**) was significantly easier to prepare than the amine precursor used in the preparation of (**56-58**).



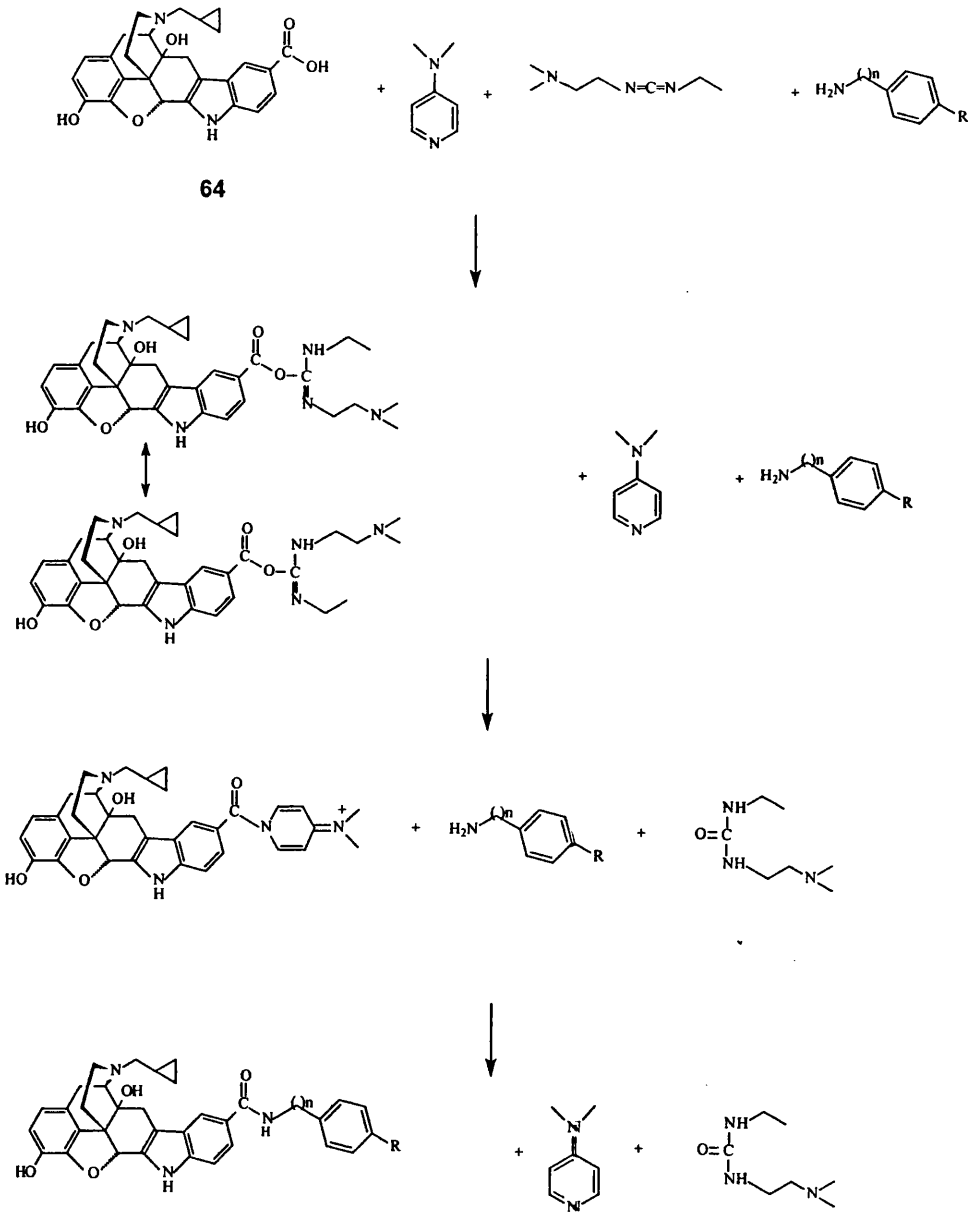
64

59

Scheme 1

The synthesis of compounds (**60-63**) was then attempted using the above methodology. The results however, were unsatisfactory, giving yields of less than 10%. Since numerous amide coupling reagents exist, we decided to investigate some alternatives. 1-[3-(Dimethylamino)-propyl]-3-ethylcarbodiimide hydrochloride (EDCI)[100] is particularly useful since it yields a water soluble side-product which can easily be separated from the product after the reaction. The yields of these reactions can often be increased by the addition of a catalytic amount of an aminopyridine, such as 4-(dimethylamino)pyridine (DMAP).

We therefore attempted the synthesis of compounds (**60-63**) by stirring carboxylic acid (**64**), EDCI, DMAP and the required amine in the presence of triethylamine at room temperature for 12 hours (**scheme 2**). The crude products were further purified by preparative thin layer chromatography [$CH_3OH/CH_2Cl_2/NH_4OH$ (20:80:1)] with typical yields of 9-22%. Unreacted amine and decomposition products could also be identified from the reaction mixture. Although the yields are very poor for this series of compounds, the reactions were not optimised since in each case, enough material for pharmacological testing was obtained after one reaction.



64

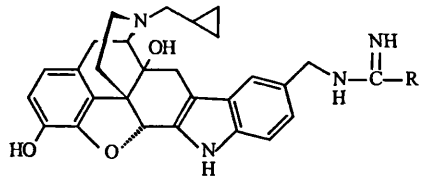
60 n = 1, R = H
61 n = 2, R = H
62 n = 4, R = H
63 n = 1, R = OMe

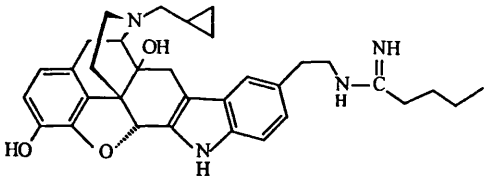
Scheme 2

2.2 AMIDINE SUBSTITUTED LIGANDS

2.2.1 DESIGN RATIONALE

As mentioned in the Introduction section, Portoghese *et al.*,[72] described a series of amidine compounds which showed good selectivity for the κ-receptor (**65-69**). It was shown that κ-selectivity increased as the length of the alkyl side chain increased. Additionally, as a result of decreased affinity for the δ-receptor, greater κ/δ-selectivity was shown for the branched alkyl derivative (**69**) than for the straight chain analogue (**68**).

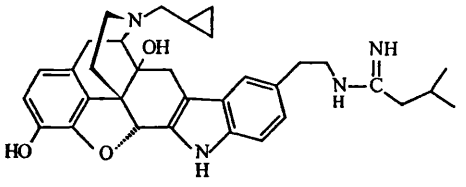




65 R = CH_3
66 R = CH_2CH_3
67 R = $(CH_2)_2CH_3$
68 R = $(CH_2)_3CH_3$
69 R = $CH_2CH(CH_3)CH_2CH_3$

70

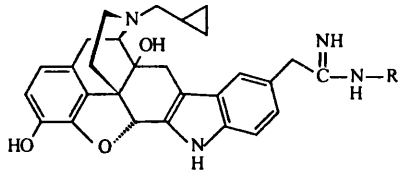
Jales *et al.*[73] were able to demonstrate increased affinity for compounds in which the basic nitrogen was one carbon atom further away from the "message" scaffold (**70**). It was not certain whether the observed increase in affinity was as a result of the greater N-N distance or as a result of the greater overall chain length. We decided to synthesise compound (**71**), where the basic nitrogen was in the position used by Jales, but branching was introduced into the alkyl side chain via incorporation of an isobutyl group. This we hoped, would combine the best features of (**69**) and (**70**), and give us greater insight into receptor/ligand interactions in the address portion of the molecule.



71

For unsymmetrically substituted amidines, the two compounds $R^1NH(C{=}NH)R^2$ and $R^1(C{=}NH)NHR^2$ are not equivalent. It was therefore of interest to see what impact this "amidine reversal" would have on the selectivity and affinity of these compounds. We decided to

synthesise a series of "reverse amidines" (**72-74**), which were otherwise analogous to the series reported by Portoghese *et al.* (**65-69**). In effect, the N-N distance for these compounds would be shorter than for the series reported by Jales. Compounds with a longer alkyl side chain were focussed on, as this was expected this to give compounds with the greatest κ-selectivity. The longest chain studied thus far was ^{n}Bu, we therefore wanted to test whether longer chains would give greater selectivity, or whether the optimum length had been reached.



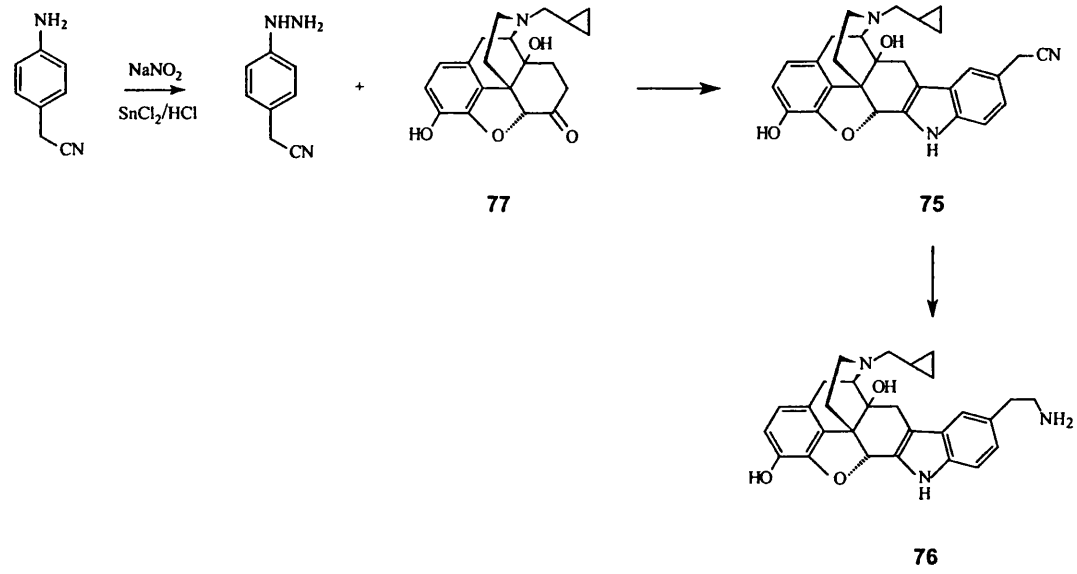
72 R = $(CH_2)_2CH_3$
73 R = $(CH_2)_4CH_3$
74 R = $(CH_2)_6CH_3$

2.2.2 SYNTHESIS

Amidines can be formed directly from the reaction of nitrile containing compounds with amines. This reaction however is limited to nitriles which are substituted by electron-withdrawing groups.[101] Alternatively, unreactive nitriles can be reacted in the presence of Lewis acids,[102] or with metal amides or amines.[103] Amidines can also be formed by the condensation of amides with amines in the presence of halogenating agents[104] or triflic anhydride.[105]

The classical approach to the synthesis of amidines, the Pinner synthesis,[106] involves the transformation of the nitrile to the alkyl imidate, which is subsequently reacted with the amine. Modifications to this reaction include the use of thioimidic esters[107] and thiophenylimidic esters[108]. Since thiols are both better nucleophiles and better leaving-groups than alcohols, the preparation of thioimidates should be possible under milder conditions. Additionally, thioimidates should be more reactive towards nucleophiles than the analogous imidates.

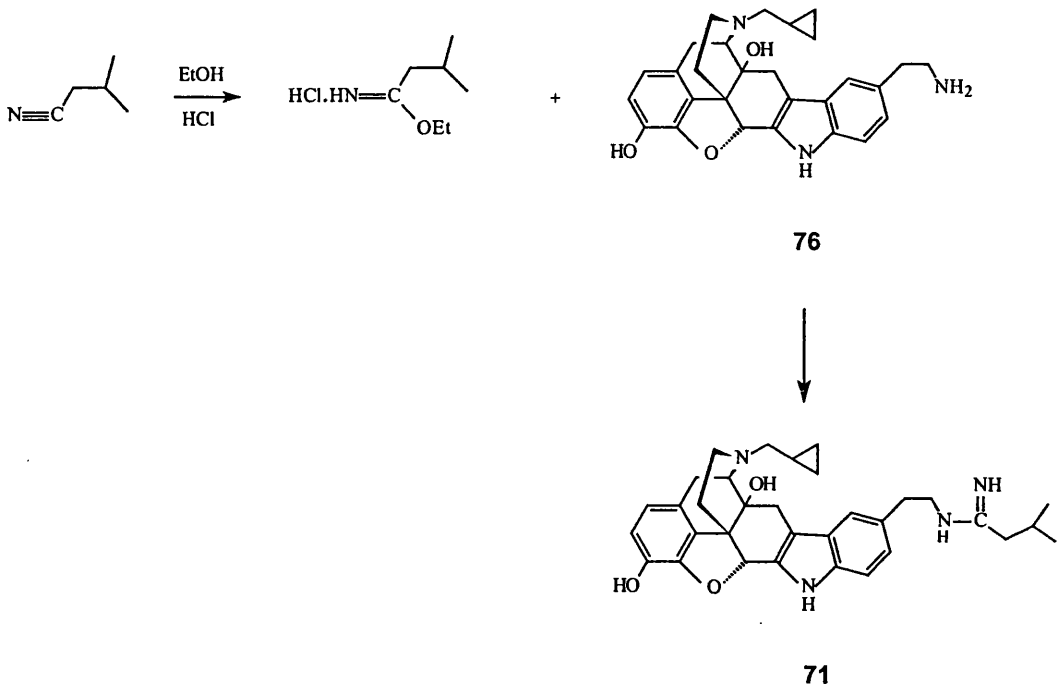
Our target compound (**71**) was very similar to that prepared by Jales (**70**) and it was decided to follow the protocol he reported. The required nitrile (**75**) and amine (**76**) intermediates were synthesised by a Fischer indole reaction between 4-hydrazinobenzylcyanide and naltrexone (**77**), followed by a Raney nickel catalysed hydrogenation (**scheme 3**).[72,73]



Scheme 3

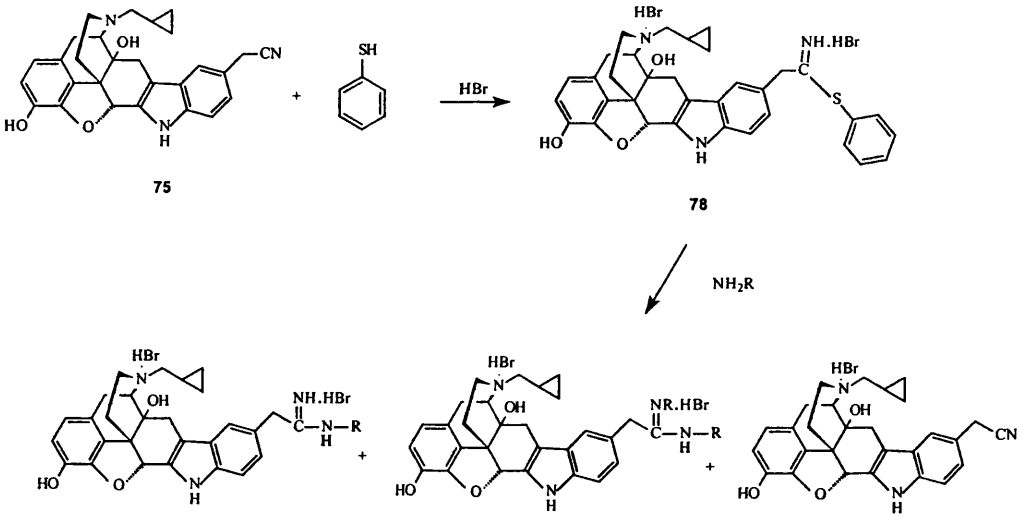
Although glacial acetic acid has previously been used as the solvent for the Fischer indole cyclisation, we found the yield to be both higher (82% vs. 46%) and more reproducible when a mixture of ethanol and 2N hydrochloric acid (5:1) was used. Raney nickel catalysed hydrogenation usually produced good yields for the reduction of nitrile (**75**) to amine (**76**), however, the yields obtained with a Raney nickel catalysed transfer hydrogenation - formic acid being the solvent - were found to be slightly superior and more reproducible. Portoghese's group later found that the addition of hydrazine hydrate to the hydrogenation mixture gave improved reproducibility.[77]

Isovaleronitrile was treated with dry ethanol and HCl gas, to give the crude imidic ester hydrochloride. The white precipitate was washed with diethyl ether, dried under nitrogen and subsequently reacted with amine (**76**) in ethanol at room temperature for 72 hours (**scheme 4**). The crude reaction mixture was purified by preparative thin layer chromatography, to give the required amidine (**71**).



Scheme 4

In order to synthesise the "reverse amidine" series (**72-74**) discussed above, the imidate of nitrile (**75**) had to be prepared before reaction with an alkylamine. Due to the reported advantages of thioimidic esters over imidic ethers,[108] it was decided to react nitrile (**75**) with thiophenol in methanol, under an atmosphere of HBr, to form the thiophenylimidic ester derivative (**78**) (**scheme 5**). The crude compound (**78**) was reacted with the appropriate amine in methanol at room temperature for 12 hours, and the product purified by preparative thin layer chromatography, giving the corresponding amidines (**72-74**) in yields of 20-30% (**scheme 5**). Apart from the desired products (**72-74**), the disubstituted amidines (**79-81**) were detected as minor products (5-10%) in each of the reactions. Additionally, unreacted nitrile (**75**) could be identified in the reaction mixture (*ca.*10%).



72 R = $(CH_2)_2CH_3$ **79** R = $(CH_2)_2CH_3$ **75**

73 R = $(CH_2)_4CH_3$ **80** R = $(CH_2)_4CH_3$

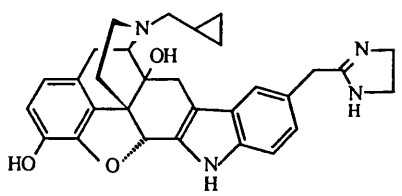
74 R = $(CH_2)_6CH_3$ **81** R = $(CH_2)_6CH_3$

Scheme 5

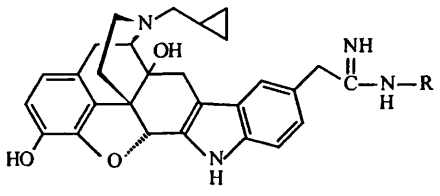
2.3 4,5-DIHYDROIMIDAZOLE SUBSTITUTED LIGANDS

2.3.1 DESIGN RATIONALE

Previously, within our group, the 4,5-dihydroimidazole (imidazoline) derivative (**82**) had been synthesised.[74] Since this is essentially a ring constrained analogue of the amidine series (**72-74**) described in the previous section, it was of interest to compare the pharmacological activities of the imidazoline and the amidines.



82

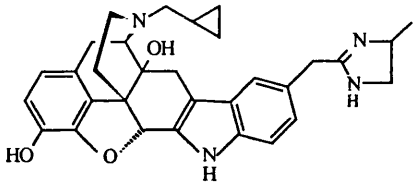


72 R = $(CH_2)_2CH_3$

73 R = $(CH_2)_4CH_3$

74 R = $(CH_2)_6CH_3$

In the amidine series, however, the presence of an aliphatic side chain could contribute to the binding *via* lipophilic interactions. For this reason, we decided to synthesise the 4-methyl- and 4,4-dimethylimidazoline derivatives (**83**) and (**84**), the methyl groups of which could interact with any lipophilic binding sites available, in a manner analogous to that of the shorter aliphatic side chain. (**83**) and (**84**) can be considered constrained ^{n}propyl and ^{t}butyl amidines, respectively.



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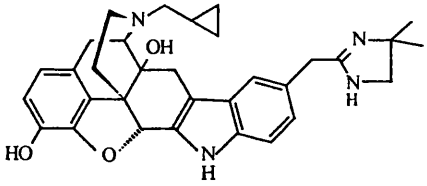
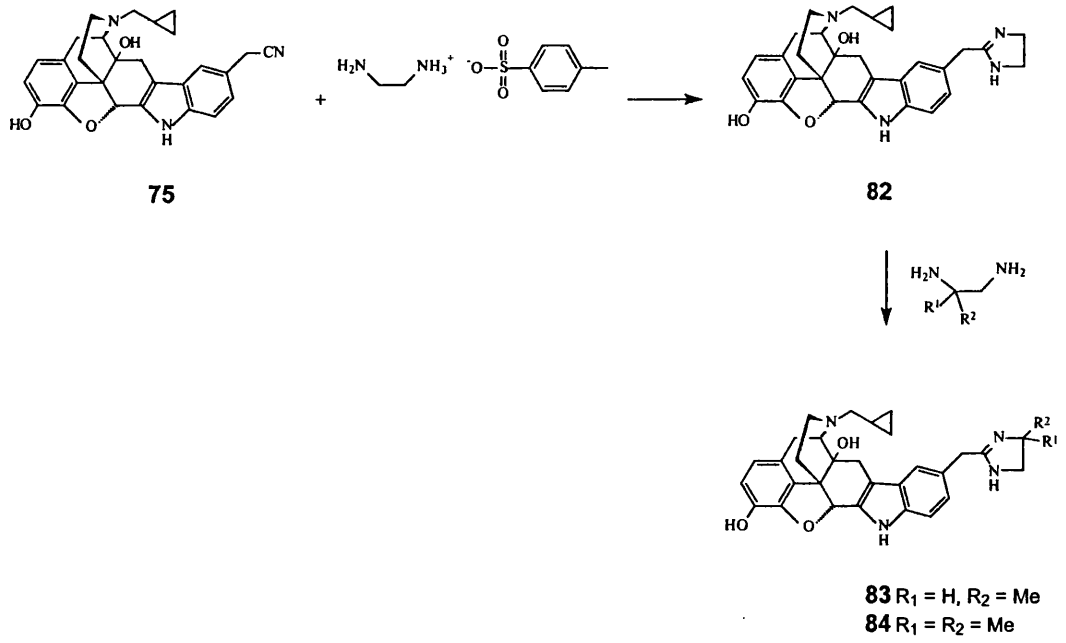


84

2.3.2 SYNTHESIS

The synthesis of 2-alkyl substituted imidazoline compounds can be achieved either by an intramolecular cyclisation reaction, or a condensation reaction. Cyclisation reactions occur with substituted (N-2-aminoethyl)amides.[109] Condensation reactions typically occur between ethylenediamine and a substituted nitrile,[110,111] carboxylic acid[112] or carboxylic acid alkyl ester.[113] Reactive nitrile groups undergo the condensation reaction with boiling ethylenediamine. Less reactive nitriles, however require temperatures higher than the boiling point of the diamine (118 °C). For this reason, the mono-acid salt of the diamine is often used. Sulfonic acid salts are favoured since they form a homogenous melt, while the reaction mixture formed with chloride salts is often heterogenous at first and the reaction consequently slower.[110]

4-Methyl- and 4,4-dimethylimidazoline derivatives reported in the literature thus far have been synthesised *via* an exchange reaction between substituted diamines and imidazolines.[114] This approach would involve two steps; first the preparation of the imidazoline (**82**), followed by the exchange reaction (**scheme 6**). Since the imidazoline compound was not prepared in a high yielding reaction (38%), this was not a favourable approach.

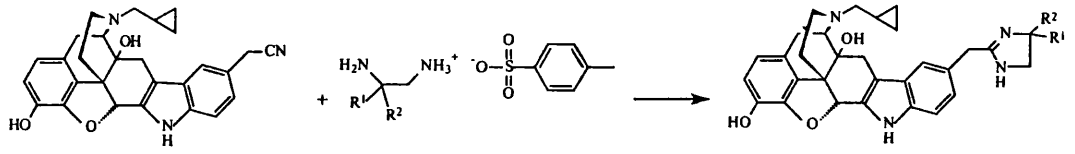


75 **82**

83 R_1 = H, R_2 = Me
84 R_1 = R_2 = Me

Scheme 6

Imidazoline (**82**) was previously synthesised *via* the reaction of nitrile (**75**) with the *p*-toluenesulfonic acid salt of ethylenediamine.[74] It has been reported that the production of amidines/imidazolines is dependant on structural factors influencing the cationic properties of the carbon atom of the cyano group and on the strength of the basic reagent.[110] Since the nitrile containing reagent would be unchanged, and methyl substituents would not be expected to have a large influence on the basicity of the diamine, it was decided to attempt a direct condensation using substituted diamines. Preparation the 4-methyl- and 4,4-dimethylimidazoline derivatives was envisaged via the reaction of nitrile (**75**) with the *p*-toluenesulfonic acid salts of 1,2 diaminopropane and 1,2 diamino-2-methylpropane, respectively.



75

83 R_1 = H, R_2 = Me
84 R_1 = R_2 = Me

Scheme 7

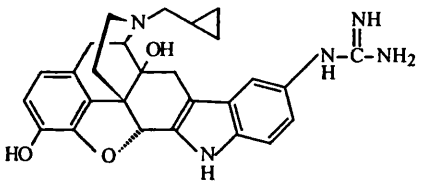
The respective diamino compounds were added to *p*-toluenesulfonic acid in ^{i}PrOH and stirred at room temperature for 30 minutes.[74] The salts were then isolated and recrystallised from petroleum ether/CH_2Cl_2, before being stirred with nitrile (**75**) (in the absence of solvent) at 160 °C for 3 hours. In each case, the crude reaction mixture was purified by preparative TLC, to yield the required products (**scheme 7**) in 5-6% yield. At the time of purification, approximately

60% starting material (nitrile compound) remained. By TLC analysis however, decomposition products were starting to appear and it was thought better to end the reaction and isolate the desired product in a low yield rather than risk further decomposition.

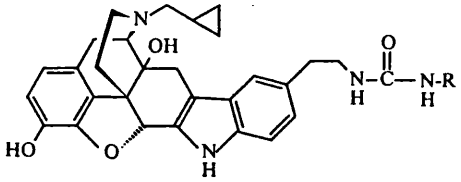
2.4 UREA SUBSTITUTED LIGANDS

2.4.1 DESIGN RATIONALE

GNTI (**47**), the most selective κ-antagonist to date, is believed to derive some of its selectivity from the highly basic guanidine moiety.[75] Although the urea functionality (pK_a *ca.* 7) is significantly less basic than the guanidine group (pK_a *ca.* 11), the relative positions of the nitrogen atoms are similar since both functionalities are necessarily planar. At this stage we were still investigating a previous finding in our group that the insertion of two methylene groups between the naltrindole "message" and the basic nitrogen "address" might improve selectivity.[74]



47



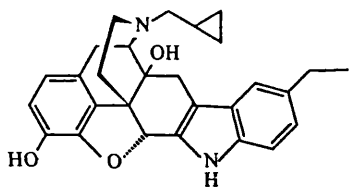
85 R = CH_2CH_3
86 R = $(CH_2)_3CH_3$
87 R = $(CH_2)_5CH_3$

We aimed therefore, to synthesise a series of 5'-ethylurea substituted ligands (**85-87**). These ligands, we hoped, would provide further information on the nature of the binding site and the level of basicity needed to provide selectivity. In a patent report by Portoghese,[72] amidinoamines and amidoamines, having the amino group ε to the message scaffold, showed greater selectivity than the analogous amidino and amido ligands. The second nitrogen atom of the urea moiety is also ε to the message scaffold. Alkyl substituents of differing lengths could be used to further probe the role of lipophilic interactions.

2.4.2 SYNTHESIS

Since we desired a urea compound substituted at one nitrogen with 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-ethyl-3,14-dihydroxyindolo[2',3':6,7]morphinan (**88**), and at the other nitrogen with an alkyl group, we were interested in procedures for the synthesis of asymmetric ureas. These include the reaction of amines with isocyanates,[115] the reaction of a carbocation with a cyanamide,[116] the reaction of an electrophile with a monosubstituted urea[117] and the reaction of an amine with an activated carbamate.[118]

Since we had amine (**76**) in hand, and alkylisocyanates were commercially available, this methodology was the most attractive.



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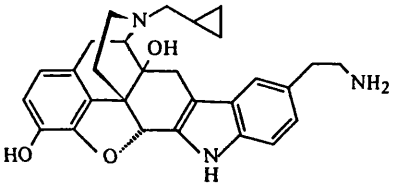
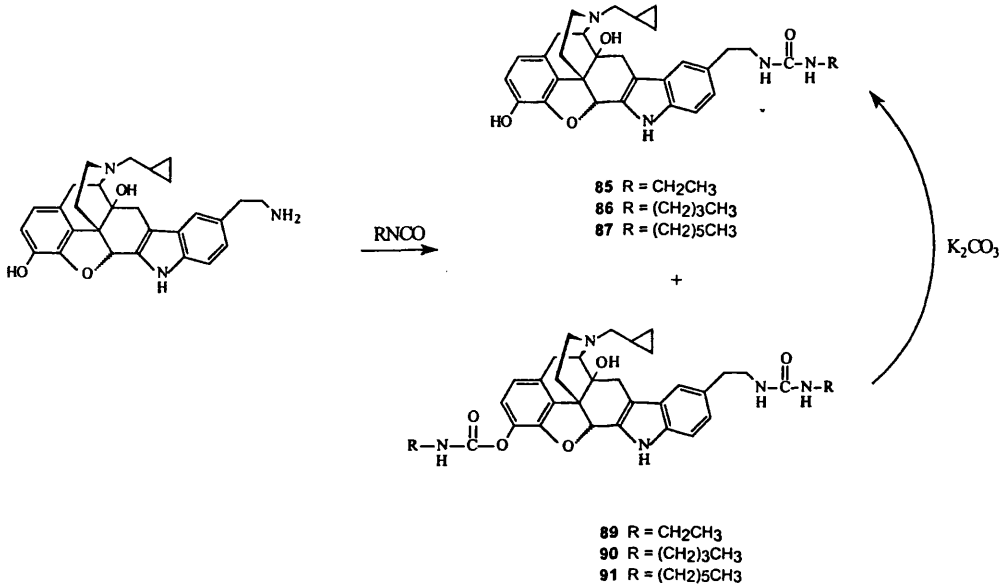


76

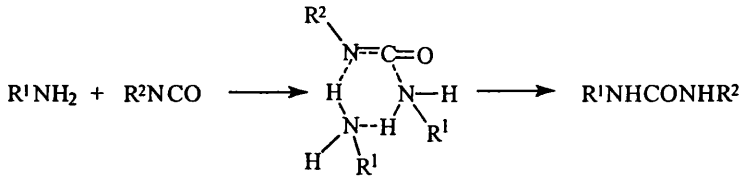
Isocyanates are able to react with both amines and alcohols. While the reaction of isocyanates with amines is fairly rapid, the reaction of alcohols is relatively slow and that of phenolic functionalities is slower still.[115] We decided to react amine (**76**) with the required isocyanates without protecting the free hydroxyl groups, since the 14-OH position is relatively hindered and the 3-OH is a phenolic hydroxyl group.

Amine (**76**) was therefore stirred with ethyl isocyanate in CH_2Cl_2 at room temperature for 5 hours (**scheme 8**). The desired urea (**85**) was isolated and purified by preparative TLC. Additionally, the urethane derivative (**89**), resulting from the reaction of the 3-OH group with ethyl isocyanate, was isolated as a by product from the reaction. Amine (**76**) was reacted with butyl and hexyl isocyanate in a similar manner, yielding typically 30-40% product (**85-87**) and approximately 10% urethane derivative (**89-91**) in all three cases. The urethane derivative could be hydrolysed to the desired product by stirring overnight with an excess of potassium carbonate (**scheme 8**).



Scheme 8

The mechanism of this reaction is analogous to an acylation in an aprotic solvent. The opening of the N=C bond can be viewed as equivalent to the departure of a leaving group.[115] Since the solvent is not able to play a role in proton transfer, a second or even third molecule of the amine is able to assist in proton transfer via a cyclic intermediate (**scheme 9**). Both tertiary amines and bifunctional catalysts, such as carboxylic acids (which can increase the nucleophilicity of the amine and assist in proton transfer), are able to catalyse this reaction. Tertiary amines are more successful at catalysing urethane formation while carboxylic acids are good catalysts for the synthesis of ureas. If the above reactions were to be repeated, the addition of a carboxylic acid catalyst may improve the yields

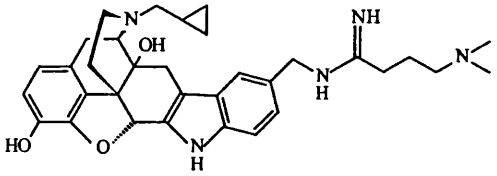


Scheme 9

2.5 SUBSTITUTED DIAMINES

2.5.1 DESIGN RATIONALE

In a patent report by Portoghese et al.[72] amidinoamines (eg. **92**) displayed approximately 2 fold greater selectivity than the analogous amidine compounds (eg. **68**) and significantly higher selectivity than norBNI. Since we had seen a certain degree of kappa selectivity displayed by amine (**76**) in GTPγS functional assays, we were interested in the effect of an alkylamino-substituent (eg. **93**) on this ligand. We hoped that these substituents would increase selectivity in a manner similar to that demonstrated in the amidinoamine series.



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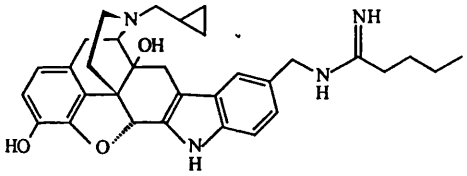

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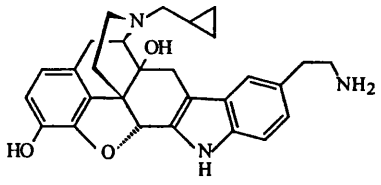

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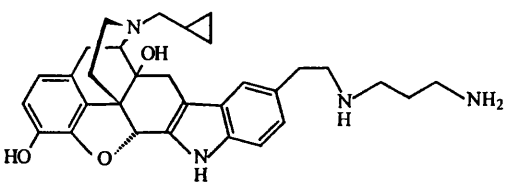
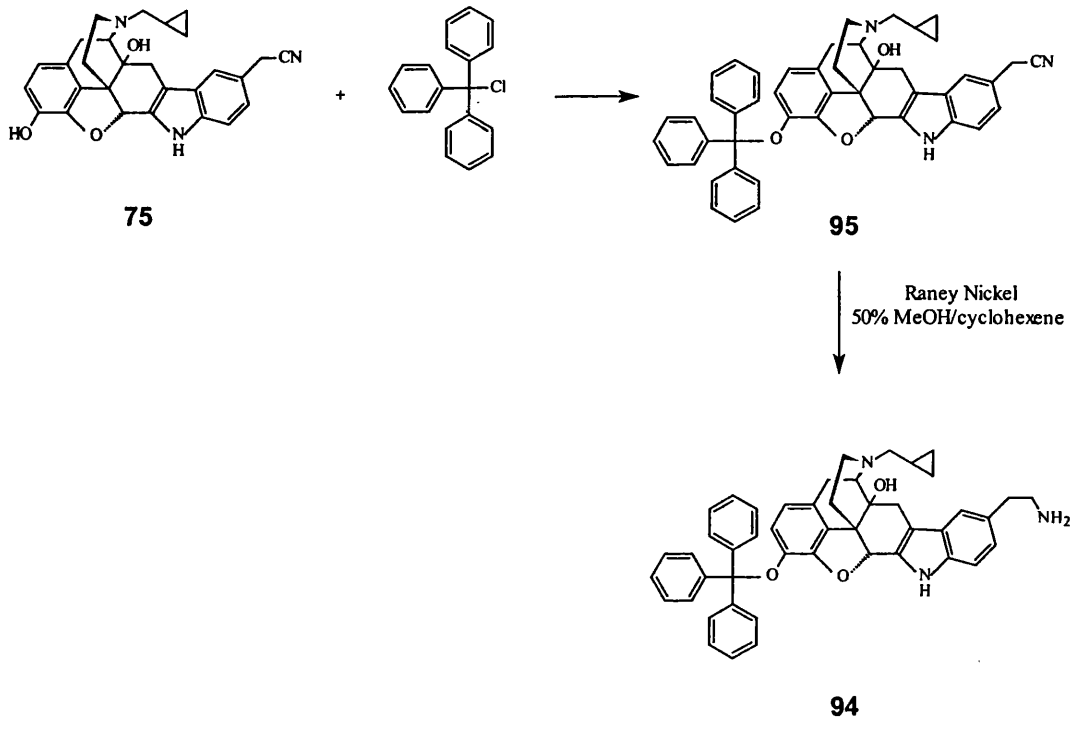


93

That the terminal amine functionality could be used as a precursor to an irreversible ligand, for example by reaction with thiophosgene to form the isothiocyanate derivative, was of additional interest to us.

2.5.2 SYNTHESIS

The initial approach involved the nucleophilic attack of amine (**76**) on an electrophile. Since the phenolic 3-OH group could also be expected to react with electrophiles, it was protected with a trityl group. This could easily be removed at a later stage under acidic conditions. Since the protection of a phenol in the presence of a primary amine could prove problematic, it was elected to protect at the nitrile stage (**75**) and then reduce to the corresponding amine (**94**). The trityl protecting group was introduced by stirring with trityl chloride in the presence of 4-dimethylaminopyridine.[119] Reduction of the trityl protected nitrile (**95**) was achieved by transfer hydrogenation using Raney nickel catalyst in 50% MeOH/cyclohexene at *ca.* 50 °C (**scheme 10**). Methanol is present in order to dissolve the compound, while cyclohexene provides the hydrogen for transfer hydrogenation. This solvent was chosen in place of formic acid (used previously to reduce the unprotected nitrile to the amine) since the trityl group is acid sensitive.

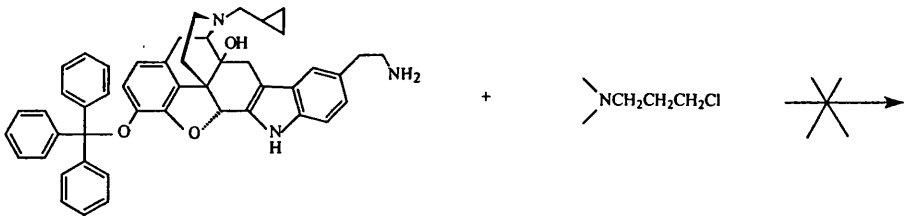


75 **95** **94**

Scheme 10

The reactions of amine (**94**) with the commercially available alkyl chloride electrophile, 3-(dimethylamino)propyl chloride (as both the hydrochloride salt and the free base), and an alkyl tosylate (**96**) were investigated. (**96**) was prepared by first reacting 3-aminopropan-1-ol with

BOC-anhydride, giving 3-(N-BOC-amino)propan-1-ol.[120] Subsequent reaction with tosyl chloride and 4-dimethylaminopyridine gave 3-(N-BOC-amino)propan-1-yl tosylate (**96**).[121] Amine (**94**), the desired electrophile and potassium carbonate were combined in dimethylformamide and stirred at RT, *ca.* 50 °C or reflux for 12 h (**scheme 11**). In each case, analysis of the mixture showed only unreacted starting material at RT, trace amounts of the desired product but mostly degradation products at 50 °C or degradation products at reflux. It was therefore decided that direct alkylation would not be the most favourable approach to this synthesis.



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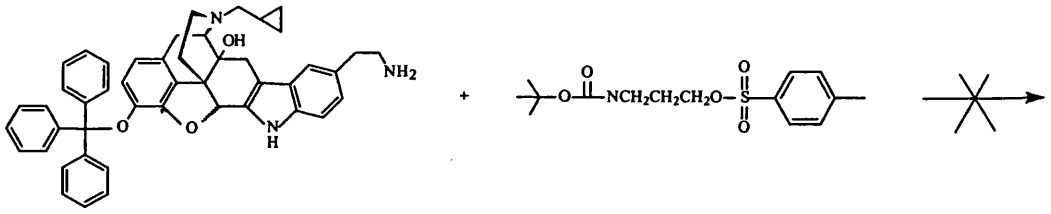
Scheme 11

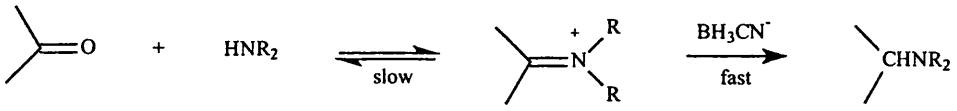


94 **96**

Scheme 12

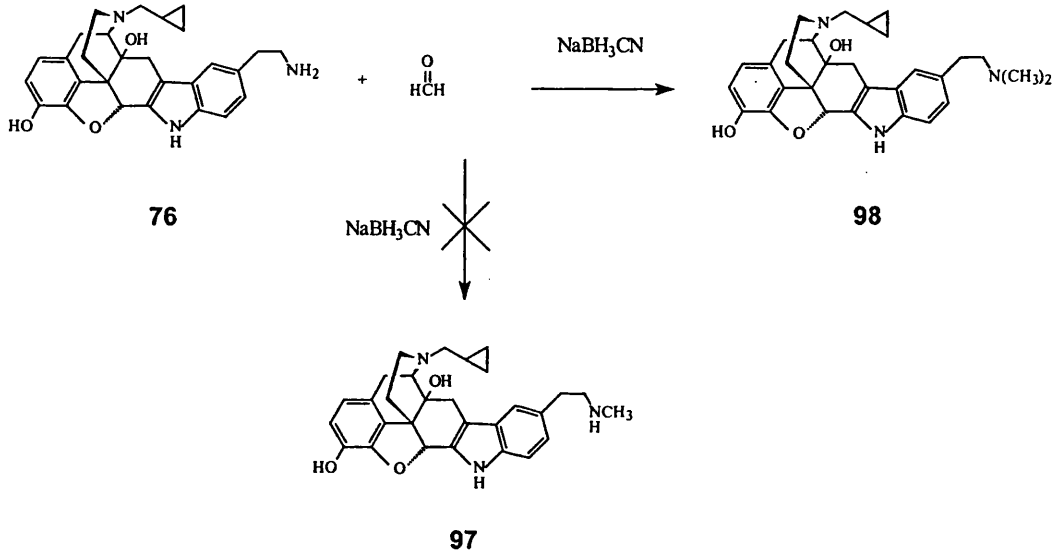
Introduction of a methyl group was then attempted via a reductive alkylation approach. This could be followed with an acylation, the amide group later being reduced to give the desired disubstituted amine derivative.

Reductive alkylations[122] involve the reaction of aldehydes or ketones with an amine, giving an imminium moiety, which can then be reduced to the secondary or tertiary amine (**scheme 13**). Formation of the imminium is the relatively slow, rate determining step, whereas the reduction reaction usually occurs rapidly. A successful reducing agent should reduce the imminium moeity without reducing the ketone or aldehyde groups. Although sodium cyanoborohydride is most often chosen as the reducing agent in reactions of this type, a wide variety of other hydride reducing agents and catalytic hydrogenation have been equally successful.[123] The amine is usually used in about five fold excess to avoid competition from the product amine.



Scheme 13

Since amine (**76**) was the most valuable reagent, it was decided to attempt the synthesis without using an excess. Instead a little over one equivalent of formaldehyde was used, with sodium cyanoborohyride as the reducing agent. Analysis of the crude reaction mixture showed none of the required product (**97**). Unreacted starting material was however detected, as well as approximately 20% of the dimethyl derivative (**98**) (**scheme 14**).



76 **98**

97

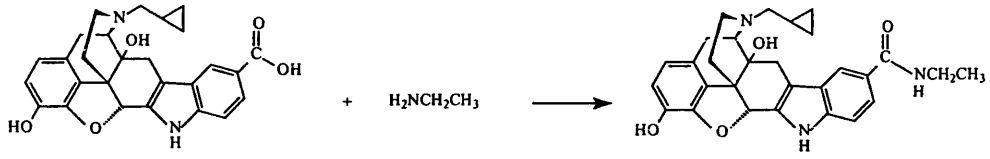
Scheme 14

Since amine (**76**) had displayed a degree of kappa selectivity,[74] we decided to prepare derivative (**98**) in greater quantity for pharmacological testing. Reacting amine (**76**) with three equivalents of formaldehyde, we were able to isolate the required product (**98**) in 57% yield.

At this stage, a report appeared in the literature in which Jones *et al.* synthesised (**98**) in good yield.[77] The major difference in our procedures was their use of methanol as solvent, the presence of excess acetic acid and allowing the reaction to proceed for 24 hours. We repeated the published procedure and found the results to be superior to our previous attempts. It is likely that the presence of the acid helps polarise the carbonyl group and generate the positive imminium species.

An alternative approach towards mono- and longer chain dialkylated products was then attempted. In the previous section, the synthesis of amide derivatives (**59-63**) is described.

Reduction of the amide functionality should yield the corresponding N-alkylamino compound. It was decided to synthesise an ethylamide derivative (**99**) using the procedures described in the previous section (**scheme 15**). If the reduction reaction proved successful, we could then synthesise the required amido amines (also mentioned in the Portoghese and Olmsted patent) and subsequently reduce them to the corresponding diamines.



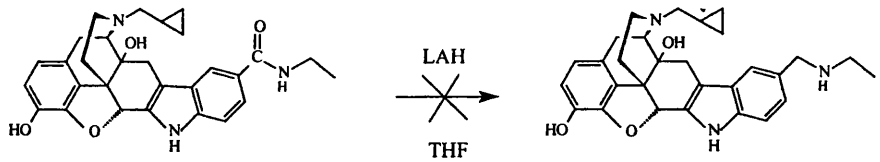
64 **99**

Scheme 15

Classically, amides have been reduced to the corresponding amines with lithium aluminium hydride (LAH) or catalytic hydrogenation.[124] Catalytic hydrogenation, however, usually requires high temperature and pressure. Since our compounds contain an indole functionality, which could be reduced under these forcing conditions, the LAH approach was preferred (reduction of an indole with LAH is much rarer).

LAH reductions are required to be performed in an aprotic solvent such as ether, tetrahydrofuran or diglyme. Even though the compound may not completely dissolve in the solvent, the reaction will usually occur as long as it is partially in solution.

Since some alkoxide formation was expected due to reaction between hydride and the hydroxy groups on (**99**), it was decided to use excess LAH for the reduction. One equivalent of ethylamide derivative (**99**) was therefore reacted with an excess of LAH in tetrahydrofuran for 12 h, at both RT and reflux (**scheme 16**). TLC analysis of the product mixture however, showed only starting material.



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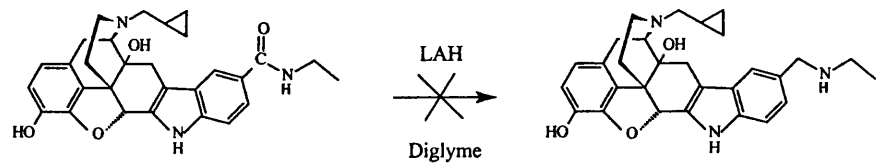


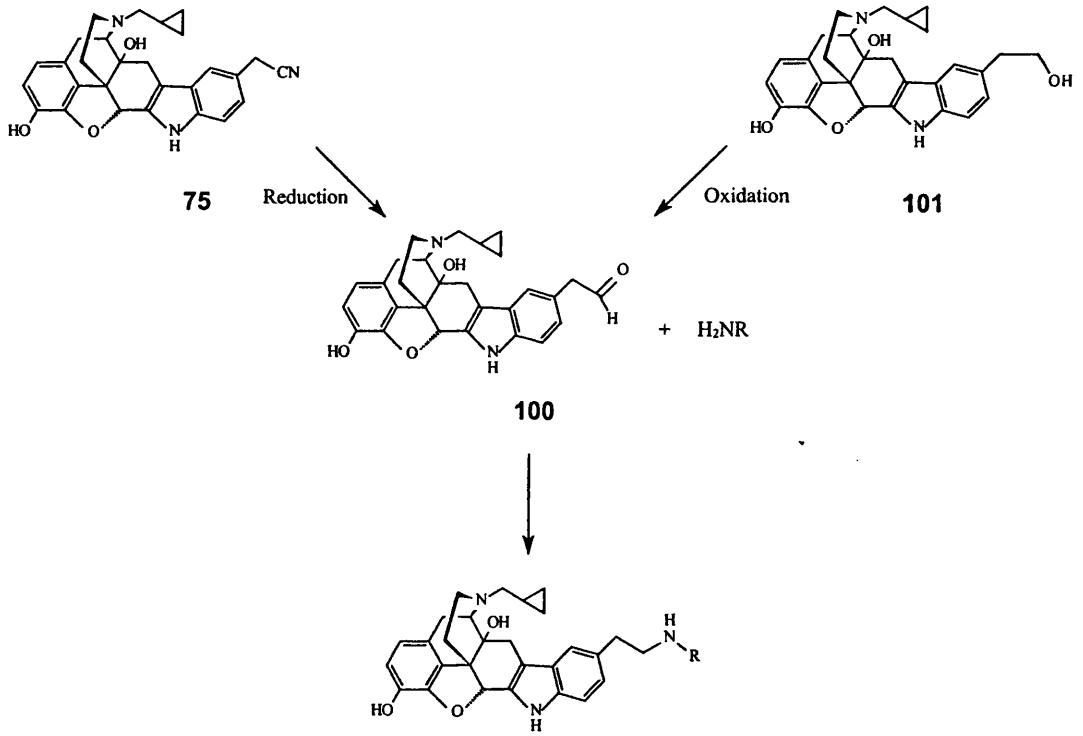
99

Scheme 16

The ethylamide (**99**) did not appear to dissolve in tetrahydrofuran and it was decided to attempt the reduction using a different solvent. One equivalent of (**99**) was therefore added to one equivalent of LAH in diglyme (**scheme 16**). Once again, the starting material did not appear to be soluble in the solvent, however, the mixture was heated at reflux for 12 hours. Subsequent TLC and mass spectrometric analysis confirmed that no reaction had taken place.

Although sodium borohydride ($NaBH_4$) alone is not able to reduce the amide group, a stronger reducing agent (B_2H_6), is formed when $NaBH_4$ is used in conjunction with other reagents such as aluminium chloride,[125] borontrifluoride[126] or iodine.[127] The reduction of (**99**) was therefore attempted with a combination of $NaBH_4$ and borontrifluoroetherate in tetrahydrofuran. The reaction was heated to reflux for 12 hours. Once again, however, TLC and mass spectrometric analysis showed only unreacted starting material.

Since a degree of success had been achieved using reductive alkylation methodology (see above), we were interested in attempting the reverse reaction, *ie.* using aldehyde (**100**) (**scheme 17**). Aldehyde (**100**) could be synthesised via reduction of the corresponding nitrile (**75**) or alternatively, *via* oxidation of the alcohol (**101**).



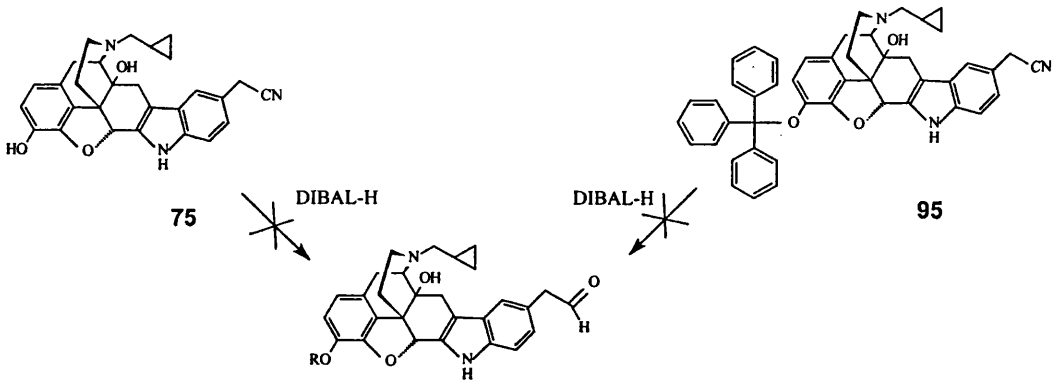
Scheme 17

Since nitrile (**75**) had previously been synthesised (**section 3.2.2**), the reduction of this compound was the favoured route to the aldehyde (**100**). Procedures for the reduction of nitrile containing compounds to the corresponding aldehydes fall essentially into two main classes.[128]

The reaction can either be achieved *via* a Stephen reduction, or *via* a metal hydride reducing agent and subsequent hydrolysis.

The Stephen reduction,[129] however, requires the presence of acid in ether or dichloromethane. Since our compound would form the salt in the presence of acid, it would not be expected to dissolve in either dichloromethane or ether and hence the reaction would not be expected to proceed satisfactorily.

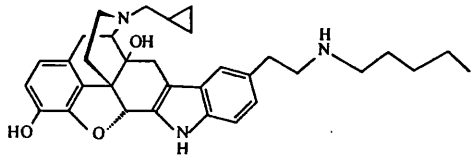
The reduction of both (**75**) and (**95**) was therefore attempted using DIBAL-H.[130] One equivalent of DIBAL-H was added to one equivalent of nitrile (**75**) or (**95**) in dichloromethane and left to stir at RT (repeated at reflux) for 12 h (**scheme 18**). Analysis upon work up however showed essentially only the respective starting material (**75**) or (**95**).



Scheme 18

Nitriles have also been converted to the corresponding aldehydes using Raney nickel catalysed transfer hydrogenation.[131] In an effort to ensure isolation of the aldehyde, we stirred nitrile (**75**) with a catalytic amount of Raney nickel in 47:47:6 MeOH/cyclohexene/H_2O at room temperature for 5 days. Water was added to the reaction mixture in order to promote hydrolysis of the imine. TLC and mass spectrometric analysis showed the formation of approximately 50% aldehyde (spot turns orange with 2,4-dinitrophenylhydrazine) and 50% unreacted starting material. If however, the reaction was left for a longer period of time, side reactions started occurring.

Attempted purification by preparative thin layer chromatography lead only to decomposition products. It was therefore decided to react the reaction mixture with pentylamine, in an attempt to isolate the required amine product (**102**). The reducing agent chosen was decaborane,[132] since this allowed the use of methanol as solvent. From the reaction mixture, we were able to isolate unreacted pentylamine, unreacted nitrile (**75**) as well as amine (**76**), formed *via* the decaborane reduction of nitrile (**75**). We were unable however, to isolate any of the desired product (**102**), imine (**103**) or the aldehyde (**100**).



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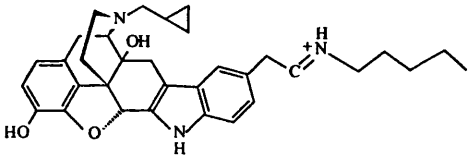
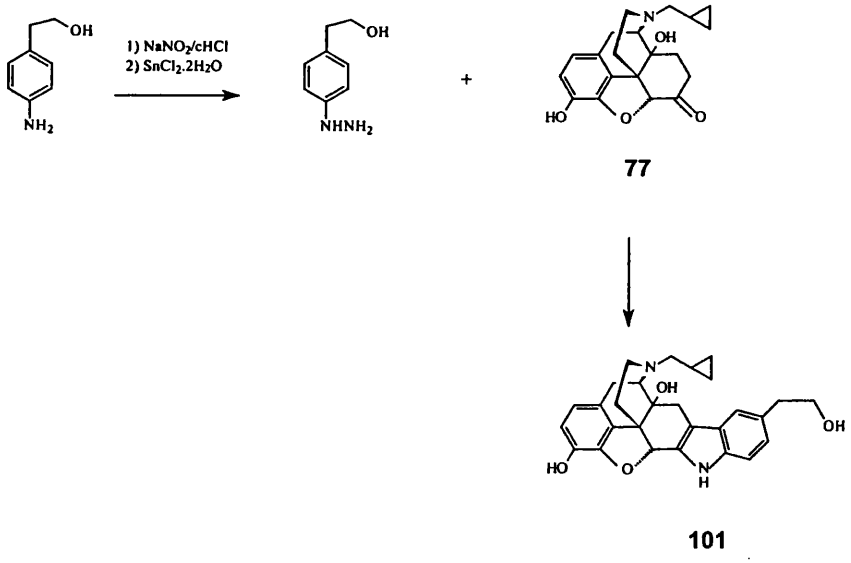


103

The oxidation of alcohol (**101**) to the desired aldehyde (**100**) was then attempted. Alcohol (**101**) was synthesised by reacting 4-hydrazinophenethylalcohol, prepared from 4-aminophenethylalcohol, with naltrexone under Fischer indole cyclisation conditions (*cf.* section 2.2.2). We were able to isolate the desired alcohol (**101**) in 8% yield (**scheme 19**). The low yield was predominantly due to poor extraction during the work up procedure and not due to competing reactions or by-product formation. Although this yield was not optimised, it would seem that this reaction would not be particularly useful as the beginning of a synthetic scheme. Nevertheless, oxidation to the aldehyde was attempted (**100**).



77

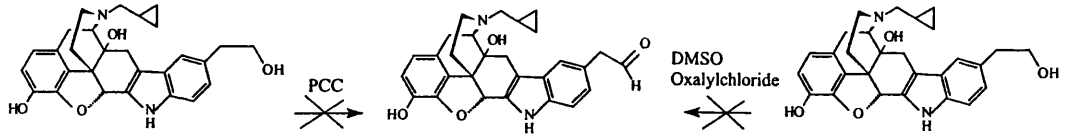
101

Scheme 19

Primary alcohols can be converted to aldehydes with strong oxidising agents, such as permanganate,[133] osmium tetroxide[134] or chromium(VI) reagents;[135] by catalytic dehydrogenation, for example with copper chromite[120] or Raney nickel;[136] with dimethylsulfoxide based reagents[137] or with hypervalent iodine reagents such as the Dess-Martin Periodinane.[138] The Oppenauer oxidation has also been used in the preparation of aldehydes, but it is more commonly used to oxidise secondary alcohols to the corresponding ketones.[120]

The oxidation of alcohol (**101**) was attempted using both pyridinium chlorochromate (PCC) and Swern conditions[139] (**scheme 20**). In neither case (at RT or reflux) could product be detected.

Swern conditions at RT or reflux, as well as PCC at RT gave only starting material, while PCC at reflux lead to the formation of degradation products.



101 **100** **101**

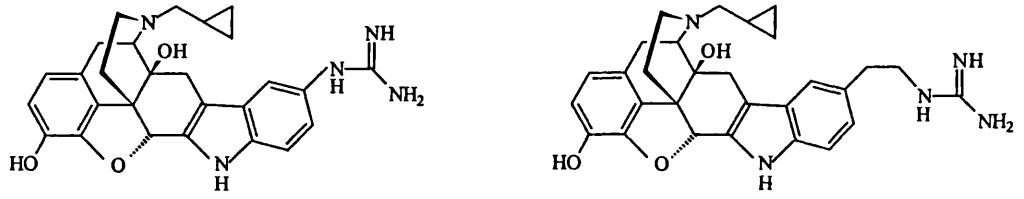
Scheme 20

At this point in the project, greater success was being achieved in the synthesis of substituted guanidines (section 2.6). The decision was made to concentrate on this new series of ligands and further attempts to reach the aldehyde were not made.

2.6 GUANIDINYL SUBSTITUTED LIGANDS

2.6.1 DESIGN RATIONALE

As discussed previously, GNTI (**47**), is the most selective κ-antagonist published to date.[75] Since amidine (**70**)[73] appeared to show greater selectivity than the analogous amidine (**68**)[72] (section 2.2.1), we sought to place the guanidine moiety in an equivalent location by preparing guanidine (**104**).

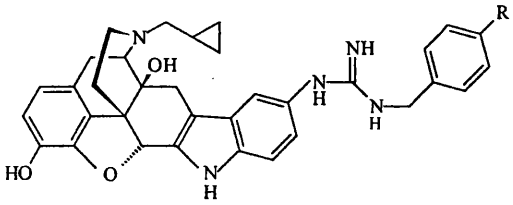


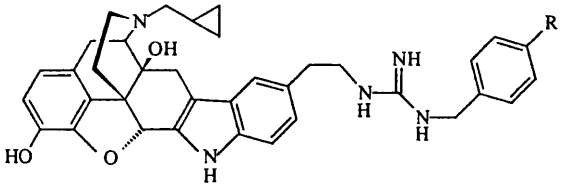
47 **104**

As a highly potent, highly selective ligand, GNTI (**47**) was an obvious candidate for further modification. Since a degree of selectivity had previously shown in the non-basic amide series,[74] we were aware that hydrophobic interactions could potentially play a role in conferring selectivity. The unsubstituted guanidinyl group provided the opportunity for introducing further substituents in the hope that greater potency and selectivity could be achieved.

With this in mind, it was also decided to investigate the influence of a benzylic substituent on the selectivity and affinity of these ligands (**105-106**). To allow a preliminary study of substituent effects on the benzyl group, *para*-substituted analogues (**107-112**) were also targeted.

Furthermore, these functionalities would allow access to potential irreversible ligands (see section 3.7)

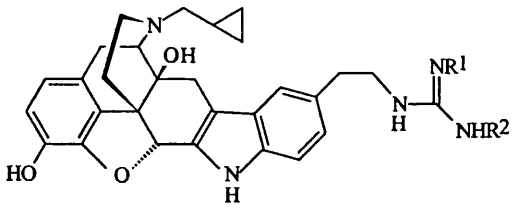


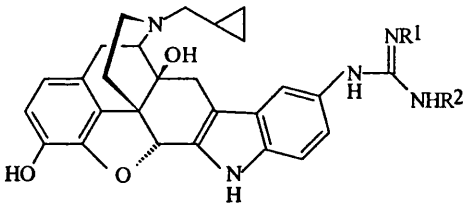


105 R = H
107 R = Cl
108 R = NO_2
109 R = NH_2

106 R = H
110 R = Cl
111 R = NO_2
112 R = NH_2

At this stage, a molecular modelling study was undertaken (section 3) and the ligands docked into a model of the kappa opioid receptor in order to identify key interactions and possible improvements that could be made. As discussed in the modelling section (3.2.7), two hydrophobic pockets for interaction between the κ-opioid receptor and the side chain of these ligands, were identified. It was thought that by adding two substituents to the guanidinyl group, we could achieve interaction with both of these pockets, thereby increasing affinity and/or selectivity for the κ-receptor. Preparation of a series of N,N'-disubstituted GNTI derivatives, for evaluation as κ-selective antagonists, was planned (**113-117**).

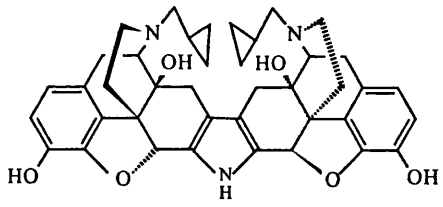




113 $R^1 = R^2 = (CH_2)_3CH_3$

114 $R^1 = R^2 = (CH_2)_3CH_3$
115 $R^1 = R^2 = (CH_2)_2CH_3$
116 $R^1 = (CH_2)_2CH_3$, R^2 = cyclopropylmethyl
117 $R^1 = CH_2C_6H_5$, R^2 = cyclopropylmethyl

Both aromatic and aliphatic substituents were chosen in order to probe this hypothesis. Of particular interest, was the preparation of compound (**117**) with a guanidinyl group possessing both a benzylic and a cyclopropylmethyl substituent. This can be seen as a stripped down version of norBNI (**40**) and comparison of the pharmacological results with those of norBNI might further support our hypothesis.



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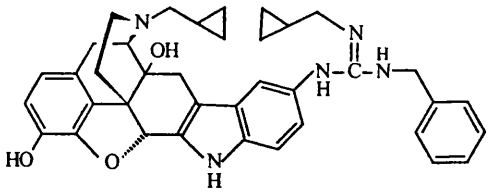
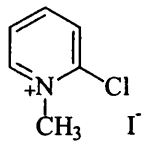


117

2.6.2 SYNTHESIS

The classical approach to the synthesis of guanidines has been the reaction of primary amines or ammonia with S-alkylisothioureas,[140] *via* the nucleophilic displacement of the alkyl mercaptan anion. The by-products formed are however often foul smelling and toxic.

Alternative procedures which have been reported include the use of carbodiimides, cyanamides and aminoiminomethanesulfonic acid derivatives.[140,141] More recently, the use of protected thiourea derivatives has greatly increased.[142] These derivatives are usually used in combination with Mukaiyama's reagent (**118**) or a thiophile such as a mercury, copper or lead salt. The solid phase application of certain of the above methods has been investigated with varying degrees of success.[143] The conversion of alcohols to protected guanidines has also been reported using Mitsonobu conditions.[143]



118

Guanidinylations are often carried out in polar solvents such as DMF or methanol, however the rate of these reactions has been reported to increase with the use of nonpolar solvents.[142]

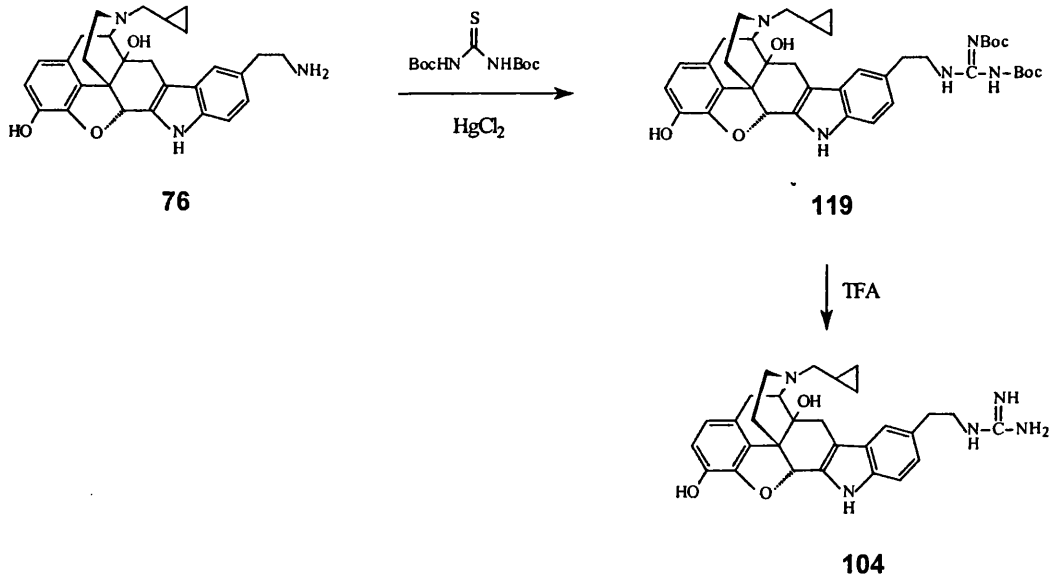
At this stage, GNTI (**47**) had only appeared in a literature communication which did not include full experimental details.[75] The formation of the guanidine group had however been based on a previously reported procedure, in which acyl guanidines had been prepared from acylthioureas.[144] Rather than a simple acyl derivative, groups such as *tert*-butoxycarbonyl (BOC) are extremely useful since they can easily be removed at a later stage.

We decided to attempt the synthesis of guanidine (**104**) using the mercury(II) chloride promoted addition of bisBOC-thiourea to amine (**76**) (as indicated for GNTI).[75] The mechanism of this reaction is not well understood. Mercury is able to form a complex with the sulfur atom, thereby

activating the carbon atom towards nucleophilic attack by the amine. Reports have been published supporting both a tetrahedral intermediate,[145] which subsequently collapses to eliminate mercury sulfide; and a carbodiimide intermediate,[146] in which the sulfur group leaves prior to nucleophilic attack by the amine. Carbodiimide intermediates have been isolated in the absence of amine (or less than 1 eq of amine). These intermediates were subsequently reacted with an amine to provide the expected guanidine.

Apart from functioning as a protecting group, the BOC group has been shown to influence the progress of the reaction - bisBOC reagents showing greater reactivity than monoBOC reagents.[146] This can be seen as a consequence of the electron withdrawing nature of the BOC group, which would promote addition of the amine. With this in mind, a variety of conjugated substituents have been investigated and found to promote guanylation. Simple N,N'-dialkyl substituted thioureas have been found to be unreactive under these conditions,[146] seemingly confirming the importance of the electron withdrawing group. Further investigations showed that a proton on each of the nitrogen groups is required for reactivity.[146] These findings would support the formation of a carbodiimide intermediate.

Adding 1.1 eq of bisBOC-thiourea and mercury(II)chloride to 1 eq of amine (**76**) and triethylamine in dimethylformamide, and stirring at room temperature for 2 hours, resulted in the formation of the desired bisBOC-protected guanidine (**119**) in 67% yield. Subsequent deprotection with trifluoroacetic acid afforded (**104**) (**scheme 21**). Purification of the deprotected product was then attempted by column chromatography. This was unsuccessful, giving a range of decomposition products. It was found however, that trituration with diethyl ether cleanly provided the guanidine product (**104**).

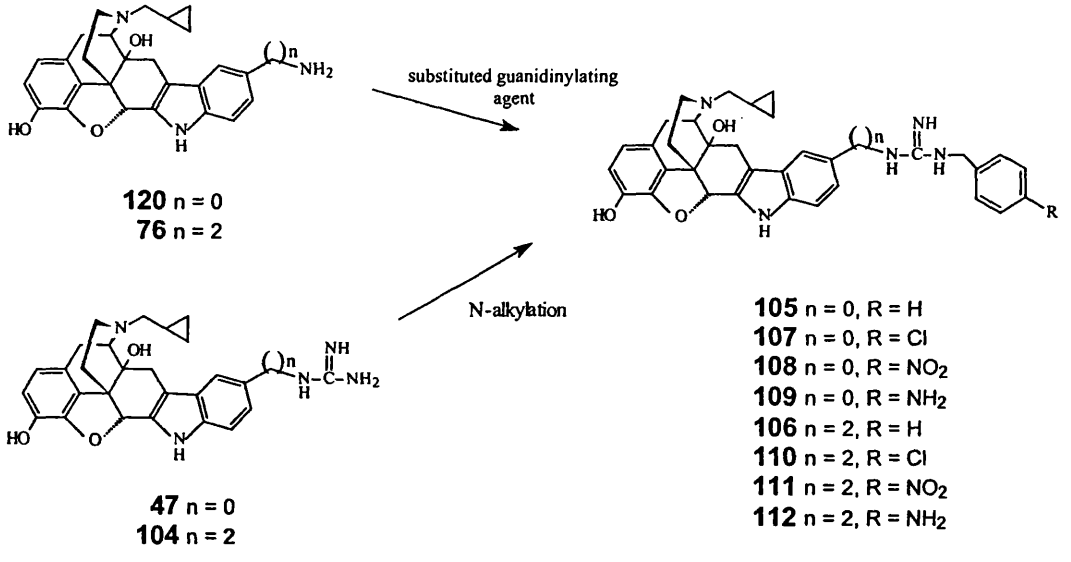


76 **119** **104**

Scheme 21

At this stage, Portoghese published a full paper[77] detailing the experimental procedure for the synthesis of both GNTI (**47**) and (**104**). Although our methods were essentially the same, slight differences in stoichiometry and reaction time existed (he added more triethylamine and used half the reaction time). It was decided to repeat the procedure reported by Portoghese, in order to compare the yields. Although no yield was quoted in the literature for compound (**104**), in our hands, more reproducible results were achieved using our procedure.

The desired benzyl-substituted guanidines (**105,107-109**) could either be synthesised via N-alkylation of the above guanidines (**47**) and (**104**)[147], or by the reaction of a substituted guanylating agent with amines (**76**) and (**120**) (**Scheme 22**).

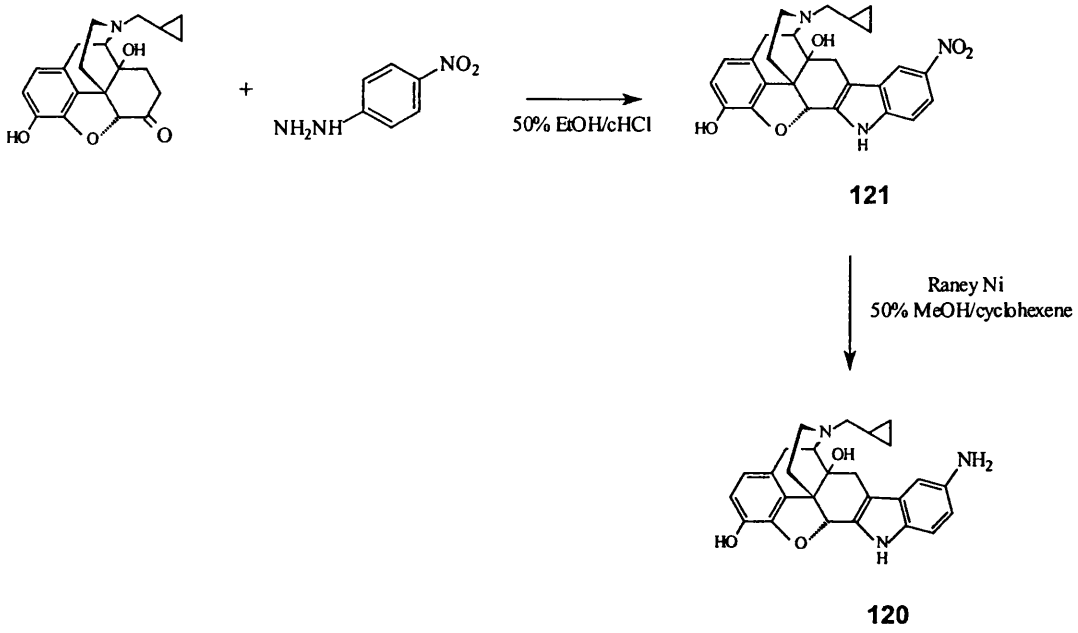


Scheme 22

Benzyl-substituted guanidinylating agents which have appeared in the literature include an N-BOC-N'-benzylthiourea[144] and N,N'-bisBOC-N-benzyl-2-methylthiopseudourea.[148] It was decided to follow the procedure using N,N'-bisBOC-N-benzyl-2-methylthiopseudourea, since the yields published were considerably higher than with N-BOC-N'-benzylthiourea, and the use of 4-substituted benzyl groups had also been investigated.

The reported synthesis of amine (**120**)[77] involved a Fischer indole reaction between naltrexone (**77**) and 4-nitrophenylhydrazine, in acetic acid at 110 °C for 7 days, giving the nitro derivative (**121**) which was then reduced to amine (**120**). Although repeating the Fischer indole procedure as described, it was found that extraction of the black, gummy residue required copious amounts of solvent and yields were consistently below 15%. The cyclisation reaction was therefore carried out in 50% EtOH/cHCl at reflux for 18 hours. Although the residue was still

found to be a black gum, compound (**121**) could be isolated, achieving consistent yields of *ca.* 30% (**scheme 23**).



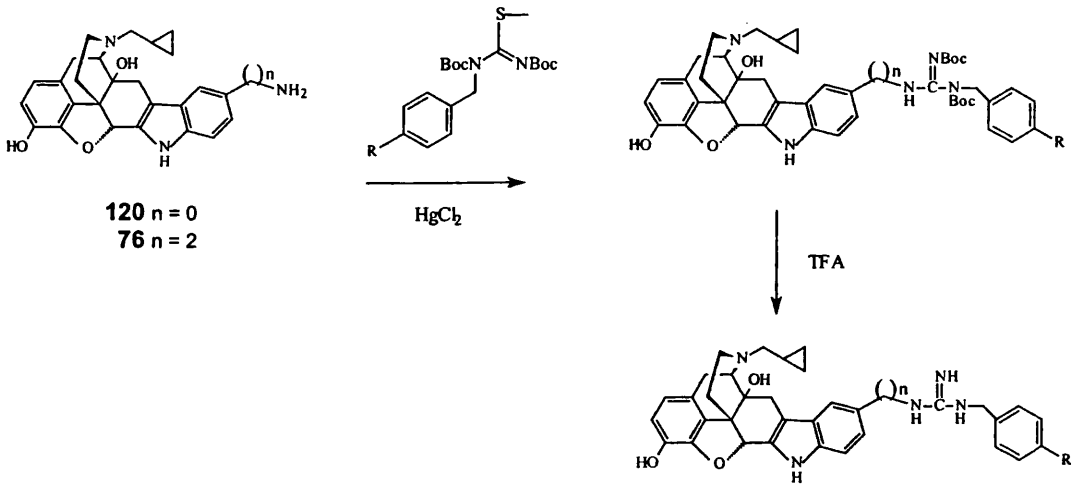
Scheme 23

In the literature,[77] the reduction of the nitro group to the amino group was achieved via Raney nickel catalysed hydrogenation in the presence of hydrazine hydrate (69% yield). Since we had previously found Raney nickel catalysed transfer hydrogenation to give more reproducible yields than standard Raney nickel hydrogenation procedures (section 3.1 and 3.2), this approach was again taken. (**121**) was stirred at *ca.* 50 °C for 7 hours in 50% MeOH/cyclohexene, with a catalytic amount of Raney nickel. The crude reaction mixture was purified by column chromatography, affording (**120**) in 44% yield (**scheme 23**).

Although amine (**76**) had previously shown lower reactivity than would be expected for a primary amine, and was considerably more hindered than the simple alkylamines used in the published procedure, we were confident that reaction with the substituted guanylating agents would be possible. The amine group in compound (**120**) can be viewed as an aniline-type moiety and would be expected to react more slowly since the electron density is delocalised over the aromatic ring.

Reaction of the appropriately substituted benzylbromide with sodium hydride and 1,3-bisBOC-2-methyl-2-thiopseudourea in DMF afforded the required 4-substituted benzylguanylating agents in good yield.

1 equivalent of 1,3-bisBOC-1-(benzyl)-2-methyl-2-thiopseudourea was added to 1 equivalent of amine (**76**) in DMF. The solution was then stirred at 60 °C for 3 hours, at which point analysis by TLC showed no conversion to the desired product. A second equivalent of the guanylating agent was added and the solution stirred at 80 °C overnight. Again, TLC analysis of the reaction solution showed only starting materials. At this stage 1.1 equivalents of mercury(II)chloride were added and the reaction stirred for a further 4 hours. TLC analysis then showed that most of the amine had been converted to the bisBOC-guanidine derivative and the reaction was quenched and worked up (**scheme 24**). The product was purified by column chromatography. Stirring the bisBOC-protected product for 12 hours in trifluoroacetic acid cleanly removed the BOC groups to give 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N'-benzyl) guanidinylethyl-3,14-dihydroxyindolo[2',3':6,7]morphinan (**106**).



120 n = 0
76 n = 2

105 n = 0, R = H
107 n = 0, R = Cl
108 n = 0, R = NO_2
109 n = 0, R = NH_2
106 n = 2, R = H
110 n = 2, R = Cl
111 n = 2, R = NO_2
112 n = 2, R = NH_2

Scheme 24

Reaction of amine (**76**) with 4-chloro- and 4-nitrobenzylguanylating agents proceeded smoothly using 2 equivalents of both the guanylating agent and triethylamine, and 1 equivalent of both the amine and mercury(II)chloride. The reaction of amine (**120**) with the guanylating agents was, as expected, more sluggish and required stirring at 60 °C for 48 hours under the same conditions.

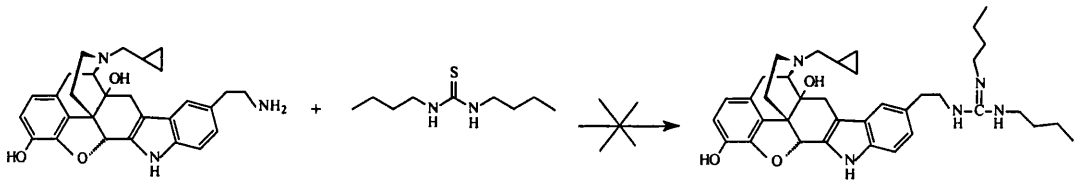
The modest yields obtained (24-44%) can partly be explained by the difficulty in purification of the guanidine products (**105,107-108** and **106,110-111**). These are almost indistinguishable

from the amine starting materials (**76** and **120**). If sufficient material for the required pharmacological tests was obtained, the remaining impure product was not purified further. In the one instance that further purification was attempted, the yield increased by about 20%.

The role of the mercury salt is not expected to be the same as for the example using the protected thiourea derivatives mentioned above, since in this case, the sulfur is alkylated. Although a mercury salt is not normally required for guanylations involving S-alkylated thiopseudoureas, a previously published article[149] has shown similar findings to ours. No mechanism has however been proposed. A black solid, presumably mercury sulfide, could be isolated from the reaction mixture once the reaction had gone to completion.

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N'-4-nitrobenzyl) guanidinyl-3,14-dihydroxy-indolo[2',3':6,7]morphinan (**108**) and 17-cyclopropyl-methyl-6,7-didehydro-4,5α-epoxy-5'-(N'-4-nitrobenzyl)guanidinylethyl-3,14-dihydroxyindolo[2',3':6,7]morphinan (**111**) were converted to their amino analogues (**109** and **112**) by Raney nickel catalysed transfer hydrogenation in 50% MeOH/cyclohexene yielding 63 and 70%, respectively. The reactions proceeded very cleanly, showing the formation of product with only small amounts of starting material remaining. The overall mass loss can be explained in terms of the affinity of the catalyst for the compound. Although the catalyst was washed thoroughly with solvent, some product remained associated with the catalyst and could not be recovered.

Literature precedents for the preparation of trisubstituted guanidines focus mainly on the reaction of N,N'-dialkyl-S-alkylisothiourea reagents with amines.[150,151] Although it has been reported that N,N'-dialkylthioureas do not form guanidines in a $HgCl_2$ promoted reaction with amines,[146] a report exists in the early literature of the formation of N,N',N''-tributylguanidine from the HgO promoted reaction of N,N'-dibutylthiourea and butylamine.[152] The HgO guanylation reaction would be expected to proceed in a manner analogous to the $HgCl_2$ promoted reaction. The synthesis of 5'-N,N'-dibutylguanydinylethylnaltrindole (**113**) was therefore attempted by reacting 5'-aminoethylnaltrindole (**76**) and N,N'-dibutylthiourea under the conditions described above for the $HgCl_2$ promoted guanidinylation reactions (**scheme 25**). It was found, in agreement with the later reports, that this reaction was unsuccessful.



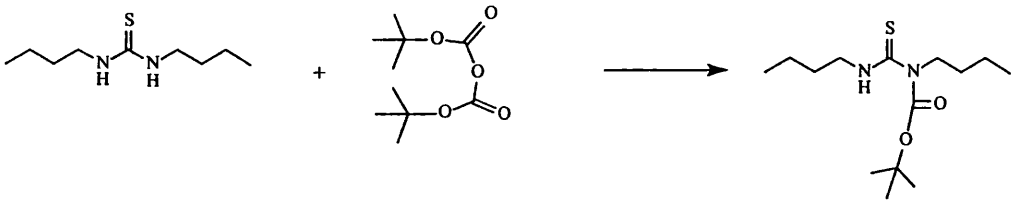
76 **113**

Scheme 25

More recently, a $CuSO_4$/silica gel-promoted procedure has shown good results in the synthesis of trisubstituted guanidines from disubstituted thioureas, including both dialkyl- and diarylsubstituents.[153]

It was decided however, to investigate the effects of introducing a BOC group into the N,N'-dibutylthiourea reagent, in a reaction similar to that used in the synthesis of compound (**104**).

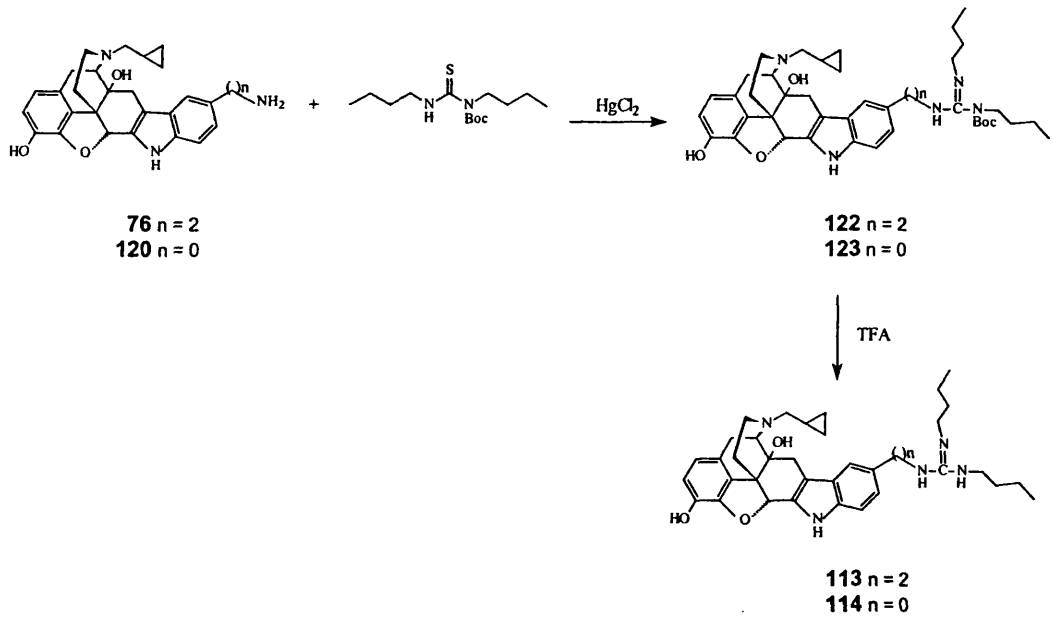
N,N'-dibutylthiourea was reacted with 2 equivalents of both BOC anhydride and sodium hydride. At this stage we were not concerned whether the mono- or bisBOC-products were obtained, as either of these reagents were expected to undergo the guanidinylation. Additionally, it was planned to remove this group after forming the guanidine. After stirring at room temperature for 3 hours, N-BOC-N,N'-dibutylthiourea was obtained (**scheme 26**).



Scheme 26

Subsequent reaction of this guanylating agent with amine (**76**) was carried out using the $HgCl_2$ promoted procedures described above. After stirring for only 5 hours, the required trisubstituted guanidine (**122**) could be isolated (**scheme 27**).

Under similar conditions, reaction of amine (**120**) with N-BOC-N,N'-dibutylthiourea gave the desired product (**123**) after 48 hours. These longer reaction times can again be explained by the lower reactivity of the aromatic amine functionality. As before, removal of the BOC-protecting groups with trifluoroacetic acid and subsequent trituration with diethyl ether gave the target compounds (**113**) and (**114**), respectively.



76 n = 2
120 n = 0

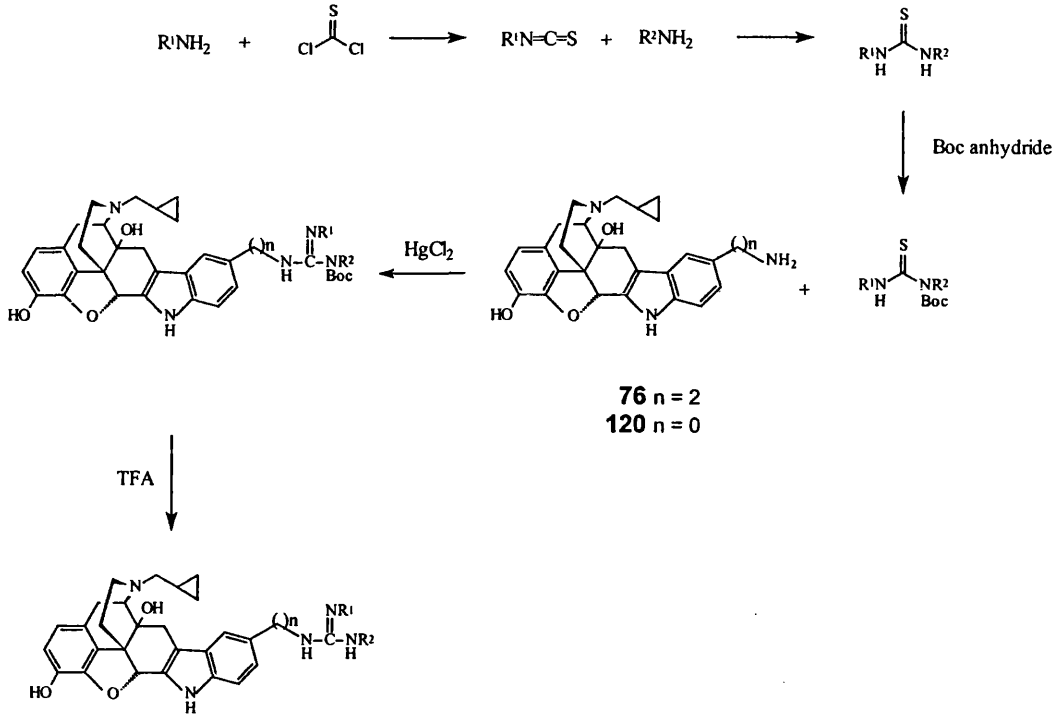
122 n = 2
123 n = 0

113 n = 2
114 n = 0

Scheme 27

A report in the literature has found that for a successful $HgCl_2$ promoted guanidinylation to occur, the thiourea should have at least one proton on each of the nitrogen groups.[146] We have, however, demonstrated that this is in fact not a strict requirement and hence for these particular examples it seems unlikely that the reaction proceeds via a carbodiimide intermediate. Under these circumstances, a tetrahedral intermediate seems more likely. Thus it would appear that the formation of guanidines by the reaction of amines with thioureas, can proceed via more than one mechanism, determined at least in part by the nature of the thiourea itself.

Modification of the substituents on the guanidine functionality, could easily be achieved by variation in the substituents on the thiourea guanylating agent. Disubstituted thioureas could easily be synthesised by the reaction of amines with either thiophosgene or CS_2 in the presence of BOP reagent. Since thiophosgene was readily available, this methodology was employed. One equivalent of the required amine was reacted with 2 equivalents of thiophosgene to give the isothiocyanate, which was isolated and checked for purity by TLC. In all cases the isothiocyanate appeared pure by TLC and was subsequently reacted with one equivalent of the second amine, to give the desired N,N'-disubstitutedthiourea (**scheme 28**). In this way, both symmetric and asymmetric N,N'-disubstituted GNTI derivatives could be synthesised. BOC protection of these guanylating agents, followed by reaction with amine (**120**) proceeded smoothly, affording the required guanidine derivatives in moderate yield (20-40%). The BOC group was cleanly removed by stirring in trifluoroacetic acid, giving the guanidine product as the bistrifluroacetic acid salt (**scheme 28**).



76 n = 2
120 n = 0

113 n = 2, R^1 = R^2 = butyl
114 n = 0, R^1 = R^2 = butyl
115 n = 0, R^1 = R^2 = propyl
116 n = 0, R^1 = propyl, R^2 = cyclopropylmethyl
117 n = 0, R^1 = benzyl, R^2 = cyclopropylmethyl

Scheme 28

2.7 NON-COMPETITIVE κ- ANTAGONISTS

2.7.1 DESIGN RATIONALE

The interest in κ-agonists as potential pharmacotherapies for cocaine abuse, yet limited information on their relative efficacies prompted us to target non-competitive κ-antagonists. As described in the introduction, irreversible antagonists can be used to determine the relative efficacy of a series of ligands, as well as providing an alternative to the use of knockout mice in determining receptor function.

To be of pharmacological use, an irreversible ligand should bind covalently and selectively to the target receptor.[154] Some ligands such as C-CAM display irreversible characteristics without actually forming a covalent bond with the receptor. These ligands are typically known as pseudo-irreversible. As mentioned previously, we sought to modify a reversible κ-antagonist in the hope that this would provide a selective, irreversible ligand devoid of agonist properties. A covalent interaction could be achieved through an alkylating agent, such as a nitrogen mustard,[38] α-haloketone, isothiocyanate[56] or Michael acceptor,[39] or by means of sulfur

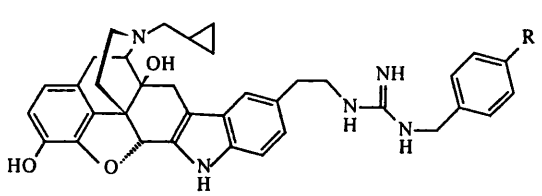
crosslinks formed with disulfide compounds.[155] The reactivity of the chosen group is important as a highly reactive moiety increases the opportunity for nonspecific binding, while a poorly reactive group may form only reversible interactions.

The isothiocyanate group is often the alkylating agent of choice in the design of non-competitive ligands, since it can be readily introduced into a molecule and the relatively small size usually does not affect selectivity to a large degree. Although this group is highly reactive towards sulfhydryl and amino functionalities, low reactivity towards water and hydroxyl groups is displayed.[156]

2.7.2 SYNTHESIS

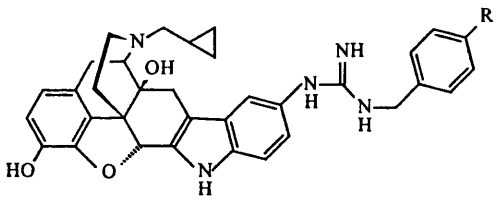
Various methods exist for introducing an isothiocyanate group. Alkyl halides,[157] acyl halides[158] or aryl diazonium compounds[159] may be reacted with a thiocyanate ion to form the corresponding isothiocyanate. In each of these cases, however, S-alkylation is a common competing reaction. Isocyanides have also been converted to isothiocyanates by treatment with disulfides and thalium(I)acetate or lead(II)acetate.[160] The most common preparation of isothiocyanates involves the reaction of a primary amine with a sulfur containing electrophile such as carbon disulfide or thiophosgene. The former reaction can take place directly in the presence of dicyclohexylcarbodiimide,[161] or alternatively, via the formation of a dithiocarbamic acid derivative. Subsequent elimination of hydrogen sulfide, yields the desired isothiocyanate.[162] The reaction of a primary amine with thiophosgene[163] procedes directly to give the isothiocyanate derivative.

The two 4-aminobenzylguanidinium compounds (**112**) and (**109**), prepared for evaluation as κ-selective antagonists in the previous section, are ideal precursors to the potential irreversible ligands (**124**) and (**125**). Pharmacological tests (see section 4) showed that in the benzylguanidinium series, the substituent in the *para* position on the aromatic ring had little influence on the selectivity or affinity of the compounds. The primary amine could be envisaged to react with either carbon disulfide or thiophosgene as detailed above. Since alcohols are known to react with carbon disulfide to give xanthates, (and compounds **112** and **109** contain both a 3-phenolic and 14-hydroxyl moiety) it was decided to use thiophosgene for the introduction of the isothiocyanate functionality.



112 R = NH_2

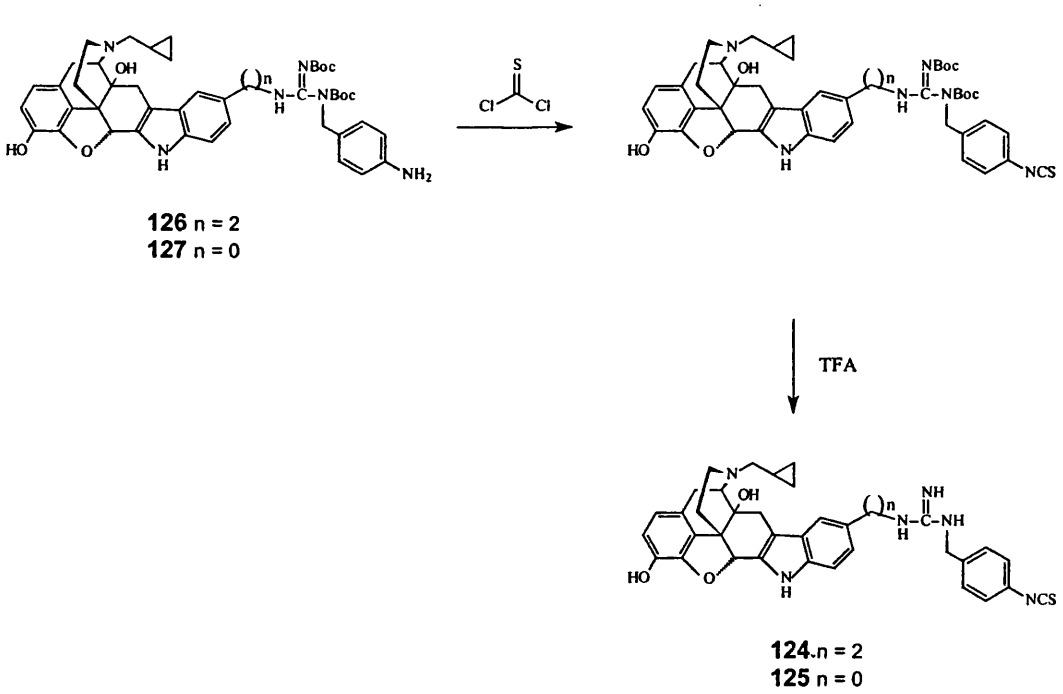
124 R = NCS



109 R = NH_2

125 R = NCS

Before removal of the BOC-protecting group, 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N'-4-aminobenzyl)guanidinylethyl-3,14-dihydroxy-indolo[2',3':6,7]morphinan (**126**) and 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N'-4-aminobenzyl)guanidinyl-3,14-dihydroxy-indolo[2',3':6,7] morphinan (**127**), were reacted with thiophosgene, to give the bisBOC-isothiocyanate derivatives in moderate yields. Subsequent treatment of (**126**) with trifluoroacetic acid removed the BOC protecting groups, affording compound (**124**). The treatment of (**127**) with trifluoroacetic acid gave a product, the mass spectometric analysis of which did not agree with the expected product (**125**). NMR analysis, however, showed that the BOC protecting groups were indeed removed. The poor solubility of both (**127**) and (**125**) has resulted in NMR spectra that are less than desirable. This reaction should therefore be repeated on a larger scale and submitted for NMR analysis in a solvent such as d^6-DMSO (**scheme 29**).



126 n = 2
127 n = 0

124 n = 2
125 n = 0

Scheme 29

3. MOLECULAR MODELLING

3.1 BACKGROUND

Numerous structure-activity relationship studies have been conducted on a wide range of opioid ligands, including both peptides and non-peptides.[1,17,164] Varying degrees of success have been achieved via this method, although the studies are normally limited to a defined series of compounds. More recently, pharmacophore models have been developed by superimposing the structures of various selective ligands.[165-168]

Most pharmacophore models have been aimed at selective ligands and hence have focussed on either the μ, κ or δ receptor. For the kappa receptor more specifically, various groups have tried to find a common pharmacophore for benzomorphan and arylacetamide ligands.[165,167] Thus far, no convincing conclusion has been reached, and pharmacophore models are often quite dissimilar, despite similar compounds being compared. Site-directed mutagenesis[169] and chimera studies[68] have suggested that different classes of κ-selective ligands interact with different residues in the receptor. For this reason, the lack of a detailed common pharmacophore is not surprising.

Recently, Filizola *et al.*[168] have attempted to find a 3D pharmacophore common to a wide variety of non-selective opioid ligands. By making use of 23, non-specific opioid ligands, a 3D model comprising four pharmacophoric centres in a defined spatial arrangement, was developed (**Fig. 2**)

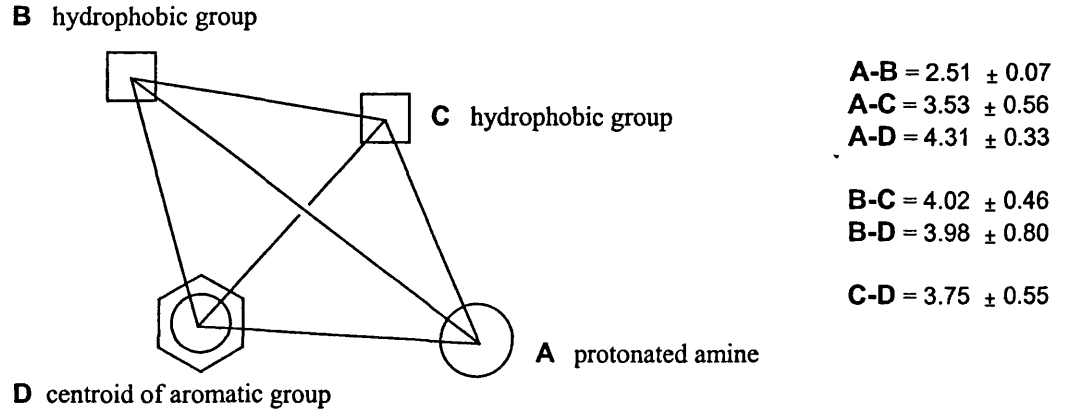


Fig. 2 3D Pharmacophore common to a wide variety of non-selective opioid ligands[168]

Obviously this method is based on the assumption that ligands can be overlapped about common pharmacophoric groups. A more robust approach would be the combination of pharmacophore modelling with receptor docking studies.[167]

The three opioid receptor types identified to date, belong to the family of guanine nucleotide-binding protein (G protein)-coupled receptors (GPCR's). The amino acid sequence of these 3 receptor types is highly conserved, with 60 - 70% identity between the different types.[165] All G-proteins share a conserved heptahelical transmembrane structure. Within the family, both the lengths of the 7 transmembrane alpha helices and the 3 extracellular loops are expected to be similar.[170]

Until recently, no high resolution structure for a GPCR had been published. A high quality 3D model for bacteriorhodopsin[171] (not a member of the GPCR family) which was based on cryomicroscopy experiments and published in 1990, however, showed an overall three dimensional structure which was remarkably similar to that proposed for GPCR's, even though the sequence homology was extremely low (<10%). It would seem likely that these receptors belong to the same functional class although not the same structural class.[172] By means of homology modelling techniques, various GPCR's have been modelled using bacteriorhodopsin as a template.[172,173]

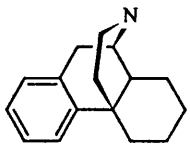
A more recently published low resolution (9Å) density map of bovine rhodopsin, a member of the GPCR family, has however shown distinct differences between bacteriorhodopsin and rhodopsin in terms of the arrangement of the seven helices.[174] Alternative approaches to modelling GPCR's which do not require the use of a template[175] have therefore also been developed. The basis of many of these methods is the comparison of the amino acid sequence of the protein with that of other GPCRs.[176] Firstly, amino acids that conserve polar character can be expected at helix-helix interfaces, while residues that conserve apolar character are more likely to be in contact with the lipids. Helical domains are most commonly predicted by means of a hydrophobicity profile. Further confirmation can be achieved by identifying the characteristic α-helix periodicity of 3.6 amino acids/turn. A Pro residue creates a specific distortion in an α-helix and hence only conserved Pro residues would be expected to form part of an α-helix. Non-conserved Pro residues could therefore indicate the boundary of a helix. The sequence divergence technique for the prediction of 3D protein structure also makes use of multiple sequence alignments. The variability profile of the aligned sequences is determined and used to predict the boundaries of transmembrane regions.

A low resolution structure of frog rhodopsin was published in 1997,[177] however in August 2000, Palczewski *et al.* published the X-ray crystallographic structure of bovine rhodopsin (PDB 1F88), with a resolution of 2.8 Å.[170] The amino acid sequence of bovine rhodopsin shows about 20 - 25% identity with that of the 3 opioid receptor types. Since we now have a high resolution structure of a receptor belonging to the GPCR family, greater confidence can be placed in receptors designed by homology modelling.

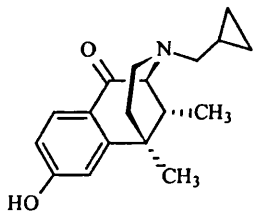
With the receptor model in hand, it is necessary to identify a potential active site for the ligand. In this respect it is often useful to look at residues that have been shown to form part of the active site in related receptors. Studies to determine the effect of mutations in the amino acid sequence have also provided useful insight into which residues influence ligand binding.

The interaction of a basic nitrogen with a conserved Asp residue has been noted for a variety of opioid ligands,[75,167,169,178-179] and is in fact a common mode of ligand interaction among G protein-coupled receptors. Since this residue is found in a region which is highly conserved among the three opioid receptor types, it is thought to form a key interaction within the "message" locus - allowing non-selective opioid recognition.[179] In the case of the kappa opioid receptor, this residue is Asp 138, which is located at the top of TM 3. Site-directed mutagenesis studies[75,178] have shown this residue to play a crucial role in the binding of opioid ligands. Further site-directed mutagenesis[167,169] and chimera studies[68] have shown that apart from a common interaction with the acidic Asp 138 residue, the 3 major classes of kappa selective ligands, the morphinans, the arylacetamides and the peptides, interact with different residues in the receptor.

For the purpose of this study we have concentrated on interactions of the kappa receptor with morphinan-type ligands (**128**). The selectivity of the morphinan antagonists was reported to lie in the interaction of a basic "address" moiety with an acidic residue in EL 3 of the kappa receptor.[68] This residue was identified as Glu 297,[69] and further confirmation was obtained by site-directed mutagenesis studies.[69,75] Portoghese *et al.* have shown that kappa affinity in the morphinan-antagonists depends on the presence of a free 3-OH group.[180] This group has been reported to interact with the acidic residue His 291.[77] Although no site-directed mutagenesis studies have been undertaken to confirm the importance of this group in the morphinans, the kappa affinity of ketazocine (KCZ, **129**) was shown to decrease upon mutation of His 291.[181] Since KCZ displays a certain amount of kappa selectivity and shares a common pharmacophore, including a free phenolic OH group, we could expect similar interactions between the ligands in this study and the receptor.



128



129

A wide variety of docking packages that show possible interactions of a ligand with a receptor, now exist. Large databases of compounds can now be screened within a reasonable amount of time in order to find compounds that may exhibit good binding. Currently, the greatest limitation to these docking packages is the scoring functions used to estimate the affinity of a

ligand for the receptor.[182] The two most important factors influencing affinity are hydrogen bonding and hydrophobic interactions.[183] Apart from finding the correct balance between each of the above, other contributing factors such as molecular weight and the number of rotational bonds should also be included when designing a scoring function. Scoring functions are presently unable to rank ligands accurately in order of increasing affinity. The best results are obtainable when the ligands involved are highly similar.

3.2 DISCUSSION

3.2.1 MODELLING THE κ-OPIOID RECEPTOR

Specific homology modelling packages now exist which match the amino acid sequence of a known receptor with the sequence of the receptor to be modelled. Due to differing lengths between conserved regions, this matching can often be improved manually. The structure of the conserved regions is then copied from that of the known receptor. In order to propose possible 3D structures for the non-conserved regions, the Brookhaven Protein Database is searched for sections similar in both amino acid sequence and length, which can then be substituted into the receptor model.

The SYBYL homology modelling package, COMPOSER,[184] was used to propose a model for the kappa opioid receptor (Appendix C). The integrity of this model, in terms of dihedral angle and bond planarity, was checked with the program, PROCHECK[185] (Appendix D).

3.2.2 PHARMACOPHORIC REQUIREMENTS FOR OPIOID RECEPTOR BINDING

The naltrindole structure is known to bind to all three opioid receptors (although affinity for the δ-receptor is significantly higher than for the μ- or κ-receptors). Compliance of this structure to the 3D pharmacophore model proposed by Filizola *et al.*[168], is demonstrated by **Fig. 3**. Since our compounds are all 5'-substituted naltrindole derivatives, it would be expected that binding to opioid receptors would occur without exception. A lack of binding could only occur as a result of steric interactions (*ie.* if the synthesised ligand were too large for the opioid binding site).

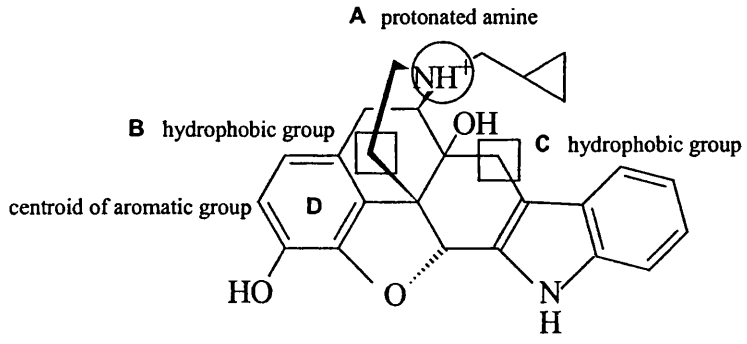


Fig. 3 The 3D pharmacophore proposed by Filizola *et al.*,[168] as applied to naltrindole

3.2.3 PHARMACOPHORIC REQUIREMENTS FOR SELECTIVE κ-OPIOID RECEPTOR BINDING

In order for a ligand to show good selectivity for a specific receptor, it is necessary to include those features that enhance affinity at that receptor, as well as those features that decrease affinity at related receptors. As discussed in section 3.1, for good κ-selective morphinan antagonist potency and affinity, N(17)H, a second basic nitrogen in the 5'-substituent and the phenolic 3-OH group are considered crucial. It has been proposed that these groups interact with Asp 138, Glu 297 and His 291, respectively.[77] While N(17)H and the phenolic 3-OH group form part of the "message" portion of the ligand, possibly contributing towards high affinity, the second basic nitrogen forms part of the "address" portion, contributing towards selectivity. In this regard, the conferring of selectivity can be explained by means of the unfavourable interactions of the basic nitrogen group with the bulky Trp 284 residue and basic Lys 303 residue, which occupy the analogous positions in the δ- and μ-receptors respectively.[77]

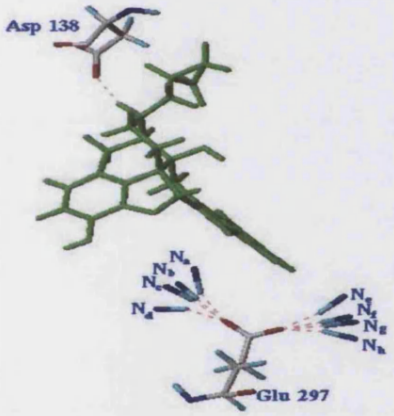
Due to the rigidity of the naltrindole structure, the relative spatial arrangement of N(17)H and the 3-OH group is fixed. The 5'-side chain, however, may contain a number of rotatable bonds, resulting in numerous possible orientations of the second basic nitrogen. It has been suggested that the optimum distance between the two basic nitrogen groups is *ca.* 11 Å.[71] This distance approximately matches the distance between Asp 138 and Glu 297. For protonated groups such as guanidines, imidazolines and amidines, the positive charge can be delocalised across the 2 or 3 nitrogen atoms. Thus, in **Table 2** we show the distance from N(17)H to each nitrogen group in the side chain of our compounds. Although the amide derivatives (**130-132,59-63**) do not have a basic nitrogen, the amide NH group is able to form a hydrogen bond with Glu 297, and are therefore included in this table. NorBNI (**40**)[62] and GNTI (**47**)[75] have been included for the sake of comparison.

Compound[a]	BU Number	Distance (Å)
130	98022	10.29
131	98023	10.27
132	98024	10.67
59	99028	9.63
60	99036	9.76
61	99037	9.65
62	20001	9.64
63	20021	9.62
133	98018	10.64, 11.68
134	98019	10.67, 12.08
135	98020	10.66, 12.07
70	98021	11.22, 13.45
71	99021	10.64, 11.93
72	99029	10.87, 9.86
73	99030	10.83, 9.79
74	99031	11.02, 9.86
82	98029	11.63, 9.79
83	99019	11.57, 9.76
84	99020	11.56, 10.01
85	20003	10.02, 11.46
86	20002	10.21, 11.78
87	99038	10.16, 9.94
76	98017	10.74
98	20032	11.18
104	20031	10.43, 12.05, 12.55
106	20022	9.34, 9.15, 11.07
110	20023	9.15, 9.34, 11.07
111	20024	9.45, 9.33, 11.20
112	20025	9.45, 9.33, 11.20
125	20028	9.36, 9.18, 11.08
105	20029	9.57, 10.19, 11.31
107	20030	9.57, 10.19, 11.31
108	01017	9.59, 10.19, 11.32
109	01018	9.59, 10.20, 11.33
124	01019	9.59, 10.69, 11.52
GNTI, **47**	-	9.58, 10.29, 11.36
NorBNI, **40**	-	11.03

[a]Compounds minimised according to the procedure described in experimental section

Table 2. Distances between N(17)H and the nitrogen groups in our compounds, GNTI and norBNI.

As can be seen from the above table, the distance between the two basic centres in the compounds varies from 9.15-13.45 Å. It is suggested that a deviation of ± 2 Å (as seen in these compounds) would not be incompatible with good κ-selectivity. There are two main reasons for this observation. Firstly, the measurements shown in the table above, and indeed for any free ligand with rotatable bonds, are dependent on the orientation of the rotatable groups. In order to measure these distances, the structures were first minimised. Although the energy of the ligand bound to the receptor should be low, it does not have to be the global minimum (usually up to 10kcal/mol above minimum). Therefore these distances could change when the ligand interacts with the receptor. Secondly, the distance given has been proposed to match the distance between Asp 138 and Glu 297. In order for strong hydrogen bonds to form, the two groups need to be 1.8-2.0 Å apart (N–H···O) and the bond angle should be as close to 180° as possible.[167] As illustrated by **Fig. 4**, various positions for the second basic group would facillitate a strong H-bond. It should also be noted that the position of the Glu 297 side chain within the receptor would also be fairly flexible.



	H···O distance (Å)	N-H···O angle (°)	N(17)-N_x distance (Å)
N_a	1.74	154.1	9.09
N_b	1.76	173.9	11.23
N_c	1.73	164.5	9.52
N_d	1.72	158.6	11.08
N_e	1.74	168.1	12.70
N_f	1.78	169.7	13.37
N_g	1.73	163.0	14.10
N_h	1.72	166.3	14.42

Fig. 4 Possible positions of NH groups able to form hydrogen bonds with Glu 297

3.2.4 SPECIFIC PHARMACOPHORE FOR DOCKING STUDIES

Brandt *et al.*[165] previously reported that in the case of KCZ, the orientation of the hydrogen of the protonated nitrogen in the kappa pharmacophore was equatorial, whereas the orientation in the X-ray crystal structure was axial. The energy of the compound with the equatorial H group was not significantly higher than that of the compound with the axial H group ($\Delta\Delta H_f$ = 0.84 kJ/mol *in vacuo*), and the inversion could therefore be expected to take place readily. Since KCZ and the morphinan-type kappa selective antagonists have been reported to bind similarly to the receptor (see section 3.1),[165] it was of interest to determine if the model of the kappa receptor accommodated the equitorial NH group for the morphinan antagonists. The naltrindole

pharmacophore, common to all the compounds, is expected to bind in the same orientation in each case. The selection of ligand used to determine the docking position and active site is therefore arbitrary.

The kappa selective, *n*-butyl amidine (**70**), with the proton in the axial position, was placed in a putative binding site in the kappa receptor, so that the N(17)H interacted with Asp 138 and the side chain amidine group formed two hydrogen bonds and a salt bridge with Glu 297 of the receptor. There was an additional interaction formed between Glu 209 and the indolic NH. Hydrophobic interactions could be seen with Val 134, Ile 135, Trp 139, Met 142, Ile 208, Trp 287, Ile 290, Phe 293, Ile 294, Leu 309, Phe 314 and Leu 318.

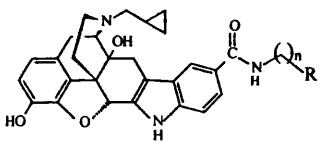
The receptor/ligand complex was then subjected to simulated annealing, molecular dynamics and numerous minimisations, however, in each case the Asp 138 residue moved away from the N(17)H so that no position could be found where the protonated N(17) could interact with Asp 138.

The structure with the best interaction energy* was then taken, the nitrogen inverted, and the ligand/protein reminimised. The resulting structure showed the retention of the above mentioned hydrophobic and ionic interactions, as well as the formation of a salt bridge between Asp 138 and the protonated N(17)H. The resultant interaction energy was about 5 kcal/mol lower, indicating a better docking position.

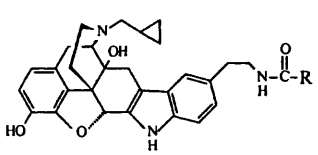
3.2.5 COMPOUNDS TO BE DOCKED

In order to gain maximum benefit from the modelling studies, we needed to investigate the docking of numerous ligands to the receptor model. It was therefore decided to make use of the ligands already described in this thesis (**59-63,71-74,76,83-87,98,104-107,110-112,125**), as well as ligands previously synthesised within our group (**70,82,130-135**)[74] and the prototypical opioid κ-antagonist, norBNI (**40**).[62] All compounds docked were antagonists with affinities in the range of 0.3-22.0 nM at cloned human κ-receptors transfected into Chinese Hamster Ovary cells (*cf.* sections 4.4 and 5.5.1).

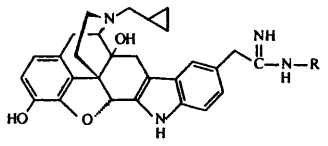
* Interaction energy cannot be used as a quantitative measure of the affinity of very different ligands for a receptor since changes in entropy and solvation are not taken into account. It is however useful for comparing different binding orientations of highly similar ligands in a receptor



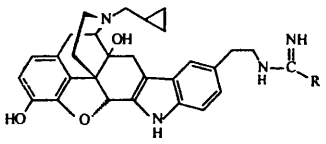
59 R = CH_3, n = 6
60 R = C_6H_5, n = 1
61 R = C_6H_5, n = 2
62 R = C_6H_5, n = 4
63 R = $C_6H_4OCH_3$, n = 1



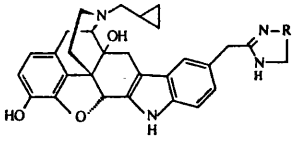
130 R = CH_2CH_3
131 R = $(CH_2)_2CH_3$
132 R = $(CH_2)_3CH_3$



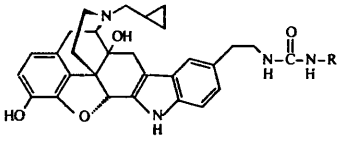
72 R = $(CH_2)_2CH_3$
73 R = $(CH_2)_4CH_3$
74 R = $(CH_2)_6CH_3$



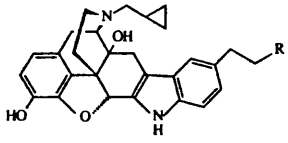
133 R = CH_3
134 R = CH_2CH_3
135 R = $(CH_2)_2CH_3$
70 R = $(CH_2)_3CH_3$
71 R = $CH_2CH(CH_3)_2$



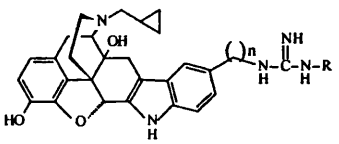
82 R = CH_2
83 R = $CHCH_3$
84 R = $C(CH_3)_2$



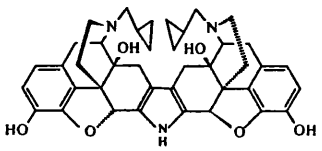
85 R = CH_2CH_3
86 R = $(CH_2)_3CH_3$
87 R = $(CH_2)_5CH_3$



76 R = NH_2
98 R = $N(CH_3)_2$



105 n = 0, R = benzyl
107 n = 0, R = *p*-chlorobenzyl
104 n = 2, R = H
106 n = 2, R = benzyl
110 n = 2, R = *p*-chlorobenzyl
111 n = 2, R = *p*-nitrobenzyl
112 n = 2, R = *p*-aminobenzyl
125 n = 2, R = *p*-isothiocyanato-benzyl



40

3.2.6 ACTIVE SITE IDENTIFICATION

The above compounds were docked into the κ-opioid receptor using the SYBYL package, FlexX.[183,186] This package is useful in that the conformational flexibility of the ligand is taken into account. The receptor is however kept rigid. The active site was defined by selecting residues Asp 138, His 291 and Glu 297; and including residues within a radius of 5Å. A base fragment is automatically selected and placed in the active site using an algorithmic approach based on a pattern recognition technique called pose clustering.[187] Ideally, a base fragment should be large enough to make specific interactions with the receptor, but it should also have as few rotatable bonds as possible. In our structures, the rigid naltrindole "core" makes an excellent base fragment. The remainder of the ligand is then built up incrementally from other fragments. A table was generated for each ligand, showing the 30 best docking positions (as scored by the scoring function within the FlexX program).

The various docking positions generated by FlexX showed the naltrindole core of the molecule in essentially the same position in each case. For the higher scoring docking positions, the side chain was in a position that allowed interaction between the protonated nitrogen and the acidic side chain of Glu 297. The aliphatic, terminal portion of the side chain was found to occupy one

of two hydrophobic pockets - either a pocket formed by residues in extracellular loop (EL) areas 2 and 3, namely Phe 214, Ile 208, Val 205 and Leu 309; or a pocket formed by residues in transmembrane (TM) regions 6 and 7, namely Phe 314, Ile 290, Phe 293 and Leu 309.

In reality, both the receptor and ligand can display a certain amount of flexibility. In addition, FlexX does not take unusual bond angle restrictions into account, *ie.* the planarity of the amide bond was not maintained. The structures could therefore often be improved by inspection, manual manipulation and minimisation of the receptor/ligand complex.

Based on the FlexX generated structures, we were able to optimise the docking position for the naltrindole portion of the molecule. An ionic interaction could be seen between N(17)H and Asp 138 (1.90 Å , 132.3°), a hydrogen bond between the phenolic hydroxyl group and His 291 (2.49 Å , 147.5°) and a hydrogen bond between the pyrrole NH group and Glu 209 (2.16 Å , 150.1°) (**Fig. 5**). Furthermore, hydrophobic interactions could be seen with residues Val 134, Ile 135, Tyr 139, Met 142, Leu 212, Trp 287, Ile 290, Ile 294, Ile 316 and Leu 318 (**Fig. 6**).

Fig. 5 Ionic interactions and hydrogen bonds between naltrindole and the κ-opioid receptor

Fig. 6 Hydrophobic interactions between naltrindole and the κ-opioid receptor

3.3. DOCKING AT κ-OPIOID RECEPTOR - RESULTS AND DISCUSSION

The naltrindole core of the molecule and most of the receptor residues were then fixed as aggregate, leaving only the 5'-side chain and surrounding hydrophobic groups for manipulation. In this way, we sought to determine the best position for each side chain. Since docking of the naltrindole portion was identical for all these structures, we hoped to find quantitative relationships between the K_i at the κ-receptor and the interactions of the 5'-side chain. Scoring the receptor/ligand interactions was accomplished using both the scoring package "SCORES" (K_D)[188], and by determining the interaction energy (*cf.* section 3.2.5).

At this stage only at the κ-receptor was being studied. Since interactions with the μ- and δ-receptors were not investigated, the results obtained relate only to the affinity of the ligands for the κ-receptor but say nothing about the selectivity. Below, descriptions of the interactions between the ligands and the receptor are given, as well as possible explanations (as seen from ligand/receptor interactions) for the observed pharmacological results. Since the docking of the naltrindole portion is constant, the descriptions focus on the interactions of the 5'-substituent. The ligands have been grouped together according to their various 5'-side chains, since comparison of the interactions is only valid for highly similar ligands (as pointed out previously for interaction energy).

3.3.1 ALKYL AMIDES (130-132)

The NH of the amide group, although unable to form ionic interactions, is able to form hydrogen bonds, due to hydrogen bond donor capabilities. In the case of these ligands, however, no such hydrogen bonds could be seen between Glu 297 and the NH group. **Table 3** details the lipophilic interactions that could be seen for these compounds.

Compound	Residue	Interaction	Residue	Distance (A)	Angle (°)	Interaction
130	Phe 293, Leu 309, Phe 314	Hydrophobic	Glu 297	-	-	-
131	Ile 293, Phe 293, Leu 309, Phe 314	Hydrophobic	Glu 297	-	-	-
132	Ile 293, Phe 293, Leu 309, Phe 314	Hydrophobic	Glu 297	-	-	-

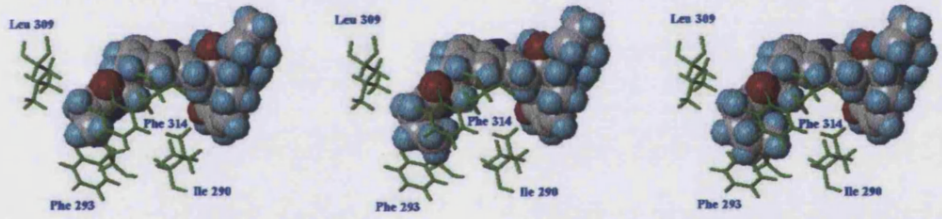
Table 3 Interactions between alkyl amides (**130-132**) and the κ-receptor

Compound	E-Total[a] (kcal/mol)	E-Receptor[b] (kcal/mol)	E-Ligand[c] (kcal/mol)	E-Interaction[d] (kcal/mol)	K_D[e]	K_i[f] (nM)
130	-196.4	-283.8	143.2	-55.7	8.93	1.57
131	-198.2	-283.1	143.1	-58.2	9.47	0.85
132	-198.1	-282.9	143.3	-58.5	9.47	0.68

[a]Total energy of the receptor/ligand complex, [b]Energy of the receptor without the ligand, [c]Energy of the minimised ligand, [d](E-Total) – (E-receptor) – (E-Ligand), [e]results from scoring package "SCORE", [f]*cf.* section 4

Table 4 Interaction energy, K_D and K_i values for alkyl amides (**130-132**)

Pharmacological assays (K_i values) show that for this series of compounds, affinity for the κ-receptor increases in the order 130<131<132. This trend would be predicted by both the interaction energies and K_D values for these compounds (**Table 4**). Since the 5'-alkylamide side chain shows no ionic interactions or hydrogen bonds with the receptor, it is envisaged that hydrophobic interactions play an important role in the binding of these ligands. In **Fig. 7**, the increase in hydrophobic interactions, which accompany an increase in the length of the alkyl side chain, can clearly be seen.



130 **131** **132**

Fig. 7 Hydrophobic interactions between alkyl amides (**130-132**) and the κ-receptor

3.3.2 AROMATIC AMIDES (59-63)

For the amide series (**59-63**), the formation of hydrogen bonds was observed between the amide NH and both oxygen atoms of Glu 297. Although hydrophobic interactions were observed with residues Ile 293, Phe 293, Leu 309 and Phe 314 (**Table 5**), the lipophilic π-stacking interactions seen between the aromatic groups of the ligand, Phe 293 and Phe 314 were the stronger of the interactions. The heptylamide (**59**) has been included with these compounds since the amide functionality occupies the same relative position. Both the hydrogen bonds as well as the lipophilic interactions mentioned above for compounds (**60-63**) could be seen. Obviously π-stacking was not possible for (**59**).

Compound	Residue	Interaction	Residue	Distance (Å)	Angle (°)	Interaction
59	Ile 293, Phe 293,	Hydrophobic	Glu 297	2.19	151.1	H-bond
	Leu 309, Phe 314			2.27	126.3	Weak H-bond
60	Ile 293, Phe 293,	Hydrophobic	Glu 297	2.10	150.0	H-bond
	Leu 309, Phe 314			2.40	137.5	Weak H-bond
61	Ile 293, Phe 293,	Hydrophobic	Glu 297	2.22	150.9	H-bond
	Leu 309, Phe 314			2.27	128.7	Weak H-bond
62	Ile 293, Phe 293,	Hydrophobic	Glu 297	2.19	149.8	H-bond
	Leu 309, Phe 314			2.34	130.0	Weak H-bond
63	Ile 293, Phe 293,	Hydrophobic	Glu 297	2.23	146.6	H-bond
	Leu 309, Phe 314			2.24	135.1	Weak H-bond

Table 5 Interactions between aromatic amides (**59-63**) and the κ-receptor

Compound	E-Total[a] (kcal/mol)	E-Receptor[b] (kcal/mol)	E-Ligand[c] (kcal/mol)	E-Interaction[d] (kcal/mol)	K_D[e]	K_i[f] (nM)
59	-202.9	-284.5	146.0	-64.4	10.4	21.89
60	-204.0	-284.9	146.4	-65.5	9.9	10.33
61	-203.6	-284.7	148.4	-67.3	10.4	2.21
62	-200.6	-284.0	148.7	-65.3	11.0	6.18
63	-204.6	-284.6	147.9	-67.9	9.16	2.11

[a]Total energy of the receptor/ligand complex, [b]Energy of the receptor without the ligand, [c]Energy of the minimised ligand, [d](E-Total) – (E-receptor) – (E-Ligand), [e]results from scoring package "SCORE", [f]*cf.* section 4

Table 6 Interaction energy, K_D and K_i values for aromatic amides (**59-63**)

K_i values show that the affinity of the phenethylamide (**61**) for the κ-opioid receptor is greater than that of either the benzyl (**60**) or phenylbutyl (**62**) analogues. In **Fig. 8**, the π-stacking interactions between the receptor and ligands (**60-63**) can be seen from above. The overlap of the aromatic rings is clearly best for compound (**61**). Although the aromatic group in compound (**63**) does not interact optimally with the aromatic residues of the receptor, the *p*-methoxy group is able to enhance the interactions. This is evidenced by a K_i value which approximates that of compound (**61**). Although the heptylamide (**59**) is able to form hydrophobic interactions, these are not as strong as the π-stacking interactions formed with the aromatic analogues. It should also be noted however that the end of the alkyl chain protrudes beyond the receptor. At this stage it is not clear whether receptors are found individually or as dimers or trimers. It is therefore uncertain what the effect of this protrusion would be, and whether the terminal portion would come into contact with another receptor or the lipid bilayer. For these amides (**59-63**), K_i values are in good agreement with interaction energies (**Table 6**). K_D values, however, do not seem to correlate well with either the K_i values or interaction energies.

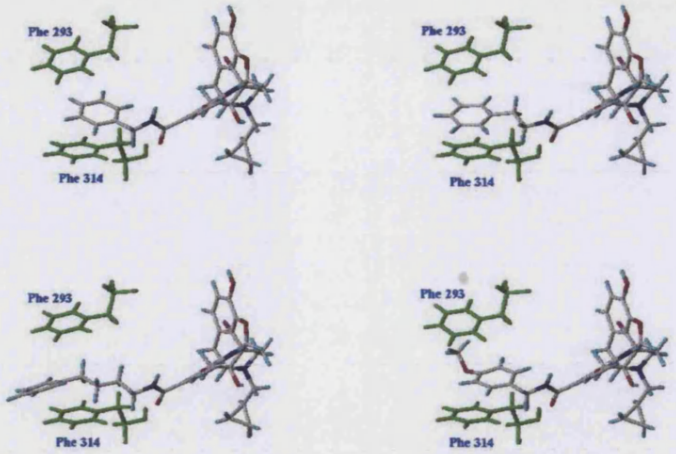


Fig 8 Hydrophobic interactions between aromatic amides (**60-63**) and the κ-receptor (clockwise from top left **60**, **61**,**63**,**62**)

3.3.3 AMIDINES (133-135,70-71)

The amidines (**133-135**,**70-71**) show an ionic bond between the protonated nitrogen group and the acidic Glu 297 residue. In addition, weak hydrogen bonds can be seen between the amidine NH group and Glu 297, and between the protonated $C=NH_2^+$ and Asp 206. Lipophilic interactions were observed with residues Val 205, Ile 208, Phe 214 and Leu 309 (**Table 7**).

Compound	Residue	Interaction	Residue	Distance (Å)	Angle (°)	Interaction
133	Val 205, Ile 208,	Hydrophobic	Glu 297	1.88	142.0	Salt bridge
	Phe 214, Leu 309			2.06	136.9	Weak H-bond
			Asp 206	2.71	133.0	Weak H-bond
134	Val 205, Ile 208,	Hydrophobic	Glu 297	1.71	140.9	Salt bridge
	Phe 214, Leu 309			1.92	134.2	Weak H-bond
			Asp 206	2.58	146.3	Weak H-bond
135	Val 205, Ile 208,	Hydrophobic	Glu 297	1.70	136.9	Salt bridge
	Phe 214, Leu 309			1.96	130.0	Weak H-bond
			Asp 206	2.60	145.9	Weak H-bond
70	Val 205, Ile 208,	Hydrophobic	Glu 297	1.69	138.8	Salt bridge
	Phe 214, Leu 309			1.96	131.7	Weak H-bond
			Asp 206	2.63	146.2	Weak H-bond
71	Val 205, Ile 208,	Hydrophobic	Glu 297	1.64	136.3	Salt bridge
	Phe 214, Leu 309			1.90	129.0	Weak H-bond
			Asp 206	2.63	148.4	Weak H-bond

Table 7 Interactions between amidines (**133-135**,**70-71**) and the κ-receptor

Compound	E-Total[a] (kcal/mol)	E-Receptor[b] (kcal/mol)	E-Ligand[c] (kcal/mol)	E-Interaction[d] (kcal/mol)	K_D[e]	K_i[f] (nM)
133	-200.2	-282.5	149.6	-67.3	ND	0.29
134	-203.1	-282.1	149.0	-70.0	ND	0.28
135	-202.3	-281.7	150.1	-70.8	ND	0.25
70	-202.3	-281.9	150.0	-70.4	ND	0.30
71	-193.8	-276.9	150.0	-66.9	ND	1.39

[a]Total energy of the receptor/ligand complex, [b]Energy of the receptor without the ligand, [c]Energy of the minimised ligand, [d](E-Total) – (E-receptor) – (E-Ligand), [e]results from scoring package "SCORE", [f]*cf.* section 4

Table 8 Interaction energy, K_D and K_i values for amidines (**133-135,70-71**)

K_i values for this series of amidines (**133-135,70-71**) show high affinity for the κ-opioid receptor. In addition, K_i appears to be independent from the length of the alkyl substituent (**Table 8**). This trend is loosely supported by the interaction energies. K_D values were not determined. Although compounds (**135,70-71**), having propyl, butyl and isobutyl groups respectively, show slightly better hydrophobic interactions with Ile 208, than do the methyl and ethyl analogues (**133-134**), no significant improvement in hydrophobic interactions can be seen as the length of the alkyl chain increases. The high affinity seen for these compounds can be attributed to the strong ionic interaction and hydrogen bonds formed between the ligand and residues Glu 297 and Asp 206. These interactions are similar for all compounds in this series (**133-135,70-71**) and are illustrated in **Fig 9**.

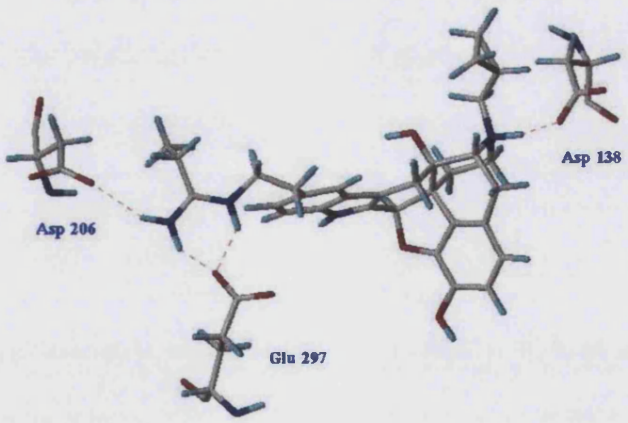


Fig 9 Ionic interactions and H-bonds between ethyl amidine (**134**) and the κ-receptor

3.3.4 REVERSE AMIDINES (72-74)

The reverse amidine compounds (**72-74**) show an ionic bond between the protonated nitrogen group and the acidic Glu 297 residue. Additionally, a weak hydrogen bond can be seen

between the NH group and Glu 297. Lipophilic interactions were observed with residues Ile 290, Phe 293, Leu 309 and Phe 314 (**Table 9**).

Compound	Residue	Interaction	Residue	Distance (Å)	Angle (°)	Interaction
72	Ile 290, Phe 293,	Hydrophobic	Glu 297	1.88	145.4	Salt bridge
	Leu 309, Phe 314			2.28	132.2	Weak H-bond
73	Ile 290, Phe 293,	Hydrophobic	Glu 297	1.83	147.9	Salt bridge
	Leu 309, Phe 314			2.38	130.0	Weak H-bond
74	Ile 290, Phe 293,	Hydrophobic	Glu 297	1.91	145.2	Salt bridge
	Leu 309, Phe 314			2.25	133.2	Weak H-bond

Table 9 Interactions between reverse amidines (**72-74**) and the κ-receptor

Compound	E-Total[a] (kcal/mol)	E-Receptor[b] (kcal/mol)	E-Ligand[c] (kcal/mol)	E-Interaction[d] (kcal/mol)	K_D[e]	K_i[f] (nM)
72	-184.4	-267.4	148.5	-65.6	ND	1.60
73	-190.0	-268.6	148.3	-69.8	ND	1.44
74	-193.7	-268.4	148.7	-74.0	ND	5.61

[a]Total energy of the receptor/ligand complex, [b]Energy of the receptor without the ligand, [c]Energy of the minimised ligand, [d](E-Total) – (E-receptor) – (E-Ligand), [e]results from scoring package "SCORE", [f]*cf.* section 4

Table 10 Interaction energy, K_D and K_i values for reverse amidines (**72-74**)

As expected, amidines (**72**) and (**73**), which are able to form both an ionic interaction and a hydrogen bond to the κ-receptor, show high affinity (low K_i) in binding assays (**Fig. 10**). Why the affinity of amidine (**74**), which interacts with the receptor in the same way as compounds (**72**) and (**73**), should be ~4 fold lower is not clear at this stage. The long alkyl chain does however extend beyond the receptor helix, as was the case for the heptylamide (**59**), which also showed low affinity. For this series (**72-74**), interaction energies are not reflective of binding results (K_i) (**Table 10**). K_D values were not determined.

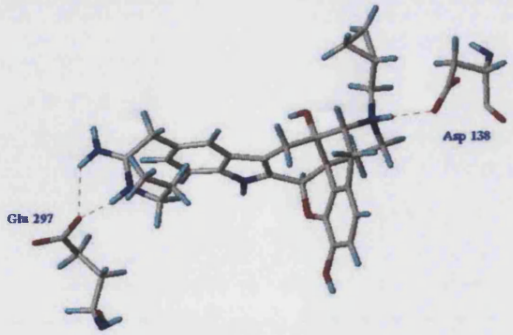


Fig.10 Ionic interactions and H-bonds between propyl reverse amidine (**72**) and the κ-receptor

3.3.5 IMIDAZOLINES (82-84)

The imidazoline compounds (**82-84**) all displayed an ionic interaction between the protonated nitrogen and Glu 297 of the κ-receptor. The unsubstituted compound (**82**) was able to form hydrophobic interactions with Val 205 and Leu 309 (**Table 11**).

Compound	Residue	Interaction	Residue	Distance (Å)	Angle (°)	Interaction
82	Val 205, Leu 309	Hydrophobic	Glu 297	1.68	141.4	Salt bridge
83	-	-	Glu 297	1.70	156.8	Salt bridge
84	-	-	Glu 297	1.70	158.0	Salt bridge

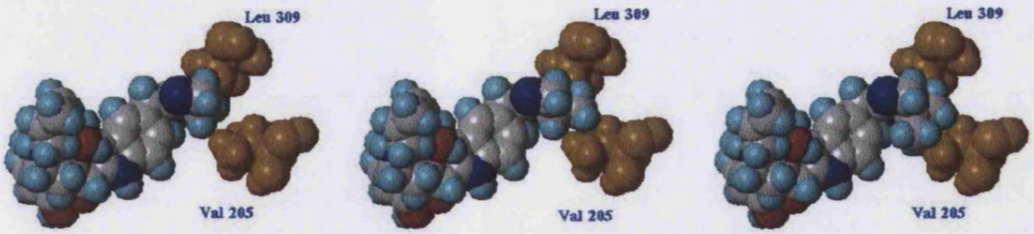
Table 11 Interactions between imidazolines (**82-84**) and the κ-receptor

Compound	E-Total[a] (kcal/mol)	E-Receptor[b] (kcal/mol)	E-LIgand[c] (kcal/mol)	E-Interaction[d] (kcal/mol)	K_D[e]	K_i[f] (nM)
82	-202.4	-285.1	149.6	-66.9	ND	0.07
83	-195.1	-284.4	149.8	-60.4	8.67	1.39
84	-186.7	-283.2	149.1	-52.5	9.08	1.97

[a]Total energy of the receptor/ligand complex, [b]Energy of the receptor without the ligand, [c]Energy of the minimised ligand, [d](E-Total) – (E-receptor) – (E-Ligand), [e]results from scoring package "SCORE", [f]*cf.* section 4

Table 12 Interaction energy, K_D and K_i values for imidazolines (**82-84**)

Once again, the formation of a salt bridge between the κ-receptor and the imidazoline ligands (**82-84**), results in low K_i values. The binding of the unsubstituted imidazoline compound (**82**) is further strengthened by the formation of lipophilic interactions between the imidazoline side chain, Val 205 and Leu 309 (typically 3.5 - 4.5Å). As substitution of the imidazoline ring increases however, (**83<84**), the methyl groups take up a position which is less than the optimum distance from Val 205 and Leu 309 (**Fig. 11**). This results in negative steric interactions and lessens the affinity of the ligands for the receptor. The trend that is evident from the binding assays (**82>83>84**), is supported by the interaction energies found for the receptor/ligand complexes for these compounds (**Table 12**). As only two of the three K_D values were determined, a valid comparison could not be made.



82 **83** **84**

Fig. 11 Hydrophobic interactions between imidazolines (**82-84**) and the κ-receptor

3.3.6 UREAS (85-87)

No ionic interactions or hydrogen bonds were seen between the urea derivatives (**85-87**) and the κ-receptor. The aliphatic portion of the side chains showed hydrophobic interactions with Ile 290, Phe 293, Phe 314 and Pro 289 (only compound **87**) (**Table 13**).

Compound	Residue	Interaction	Residue	Distance (Å)	Angle (°)	Interaction
85	Ile 290, Phe 293 Phe 314	Hydrophobic	Glu 297	-	-	-
86	Ile 290, Phe 293 Phe 314	Hydrophobic	Glu 297	-	-	-
87	Ile 290, Phe 293 Phe 314, Pro 289	Hydrophobic	Glu 297	-	-	-

Table 13 Interactions between ureas (**85-87**) and the κ-receptor

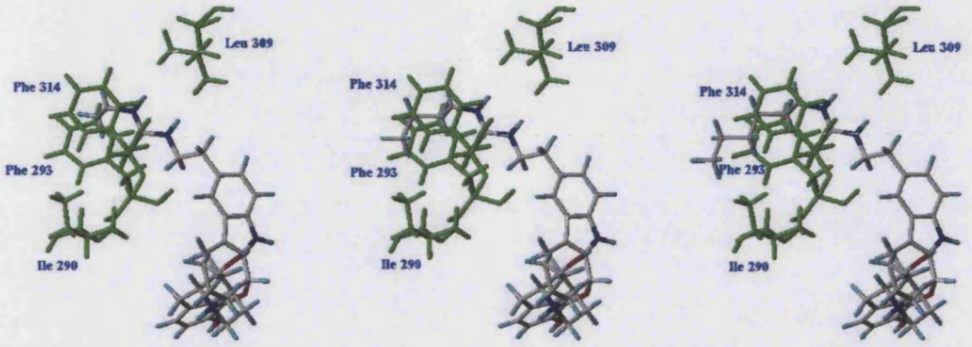
Compound	E-Total[a] (kcal/mol)	E-Receptor[b] (kcal/mol)	E-Ligand[c] (kcal/mol)	E-Interaction[d] (kcal/mol)	K_D[e]	K_i[f] (nM)
85	-199.4	-284.4	143.1	-58.0	7.90	12.32
86	-200.1	-284.2	143.3	-59.2	8.44	6.33
87	-201.6	-283.7	144.1	-62.0	8.98	8.13

[a]Total energy of the receptor/ligand complex, [b]Energy of the receptor without the ligand, [c]Energy of the minimised ligand, [d](E-Total) – (E-receptor) – (E-Ligand), [e]results from scoring package "SCORE", [f]*cf.* section 4

Table 14 Interaction energy, K_D and K_i values for ureas (**85-87**)

The affinity of the urea derivatives (**85-87**) for the κ-opioid receptor is not particularly high. Since no ionic interactions or hydrogen bonding is seen between the receptor and the ligands, the strength of the interaction is most probably dependant on the lipophilic interactions.

Lipophilic interactions are observed with Ile 290, Phe 293, Phe 314 and Pro 289 (for compound **87**). In each case, however, an NH group of the urea points toward the lipophilic residue, Leu 309 (**Fig. 12**). This causes unfavourable interactions that would lower the affinity of the ligands for the receptor. Both K_D values and interaction energies tend to agree with each other, however they do not correctly predict the results of the binding studies (K_i) (**Table 14**).



85 **86** **87**

Fig. 12 The unfavourable interaction of Leu 309 with the NH group of ureas (**85-87**)

3.3.7 UNSUBSTITUTED AND SUBSTITUTED AMINES (76,98)

The primary amine (**76**) showed the formation of a strong ionic interaction between the protonated nitrogen and Glu 297. Additionally, hydrophobic interactions could be seen with residues Phe 314 and Ile 290 (**Table 15**). Although the substituted amine analogue (**98**) also displayed the formation of an ionic interaction, it was much weaker than that for compound (**76**) due to the less than optimum bond angle. Lipophilic interactions could be seen between the ethyl group (preceding the amine functionality) and hydrophobic residues Ile 290, Phe 293 and Phe 314. (**Table 15**).

Compound	Residue	Interaction	Residue	Distance (Å)	Angle (°)	Interaction
76	Ile 290, Phe 314	Hydrophobic	Glu 297	1.61	167.0	Salt bridge
98	Ile 290, Phe 293, Phe 314	Hydrophobic	Glu 297	1.94	129.0	Weak salt bridge

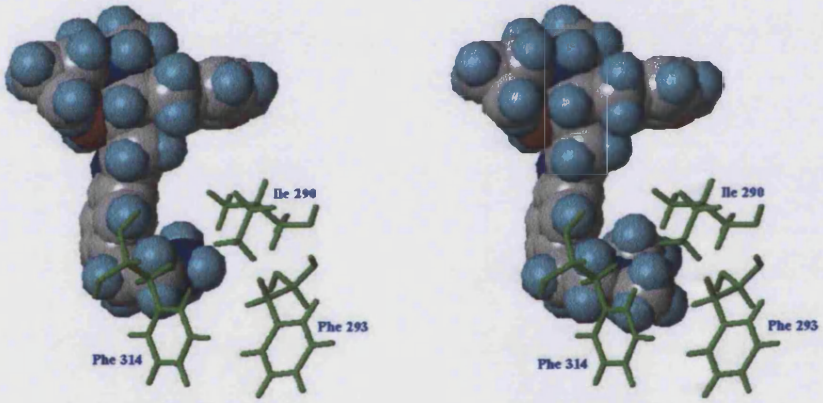
Table 15 Interactions between amines (**76,98**) and the κ-receptor

Compound	E-Total[a] (kcal/mol)	E-Receptor[b] (kcal/mol)	E-Ligand[c] (kcal/mol)	E-Interaction[d] (kcal/mol)	K_D[e]	K_i[f] (nM)
76	-200.0	-284.7	145.8	-61.1	8.14	1.08
98	-195.1	-283.6	149.5	-61.0	7.80	0.40

[a]Total energy of the receptor/ligand complex, [b]Energy of the receptor without the ligand, [c]Energy of the minimised ligand, [d](E-Total) – (E-receptor) – (E-Ligand), [e]results from scoring package "SCORE", [f]*cf.* section 4

Table 16 Interaction energy, K_D and K_i values for amines (**76**,**98**)

The affinity of both (**76**) and (**98**) for the κ-receptor was high, indicating strong ligand/receptor interactions (**Table 16**). The formation of a salt bridge between the protonated 5'-side chain and the receptor was therefore expected. Although the ionic interaction formed between the primary amine (**76**) and the receptor is stronger than that formed between tertiary amine (**98**) and the receptor, the latter shows greater affinity (lower K_i). This may be as a result of the stronger hydrophobic interactions between (**98**) and the κ-receptor (**Fig. 13**).



76 **98**

Fig. 13 Hydrophobic interactions between amines (**76**,**98**) and the κ-receptor

3.3.8 GUANIDINYLETHYL DERIVATIVES (104,106,110-112,125)

All the guanidinylethyl derivatives (**104**,**106**,**110-112**,**125**) bound to the receptor in such a way as to allow the formation of a salt bridge between the protonated nitrogen group and Glu 297 (**Table 17**). The unsubstituted guanidine (**104**) was able to form additional hydrogen bonds between the basic side chain and both Glu 297 and Asp 206. Lipophilic interactions could be seen between the side chain of (**104**) and Phe 314. The phenyl rings of derivatives (**106**,**110-112**,**125**) were able to interact with the hydrophobic residues Ile 290, Phe 293, Leu 309 and Phe 314 (**Table 17**).

Compound	Residue	Interaction	Residue	Distance (Å)	Angle (°)	Interaction
104	Phe 314	Hydrophobic	Glu 297	1.71	146.1	Salt bridge
				1.88	139.6	H-bond
			Asp 206	2.56	150.0	Weak H-bond
				2.73	144.5	Weak H-bond
106	Ile 290, Phe 293, Leu 309, Phe 314	Hydrophobic	Glu 297	1.97	148.2	Salt bridge
110	Ile 290, Phe 293, Leu 309, Phe 314	Hydrophobic	Glu 297	1.94	150.9	Salt bridge
111	Ile 290, Phe 293, Leu 309, Phe 314	Hydrophobic	Glu 297	1.94	150.5	Salt bridge
112	Ile 290, Phe 293, Leu 309, Phe 314	Hydrophobic	Glu 297	1.96	149.0	Salt bridge
125	Ile 290, Phe 293, Leu 309, Phe 314	Hydrophobic	Glu 297	1.95	149.7	Salt bridge

Table 17 Interactions between guanidinylethyl derivatives (**104,106,110-112,125**) and the κ-receptor

Compound	E-Total[a] (kcal/mol)	E-Receptor[b] (kcal/mol)	E-Ligand[c] (kcal/mol)	E-Interaction[d] (kcal/mol)	K_D[e]	K_i[f] (nM)
104	-199.4	-282.2	149.2	-66.4	8.81	0.49
106	-200.7	-283.8	154.4	-71.3	9.35	1.42
110	-202.6	-283.6	154.1	-73.1	10.1	2.41
111	-199.9	-283.5	156.8	-73.2	10.0	2.14
112	-200.6	-283.7	154.8	-71.8	9.4	0.95
125	-203.5	-283.6	154.8	-74.7	10.9	awaited

[a]Total energy of the receptor/ligand complex, [b]Energy of the receptor without the ligand, [c]Energy of the minimised ligand, [d](E-Total) – (E-receptor) – (E-Ligand), [e]results from scoring package "SCORE", [f]*cf.* section 4

Table 18 Interaction energy, K_D and K_i values for guanidinylethyl derivatives (**104,106,110-112,125**)

The high affinities of these compounds (**104,106,110-112,125**) for the κ-receptor could be expected since a strong ionic interaction is able to form between the protonated 5'-side chain and Glu 297 (**Fig. 14**). The phenyl groups are also able to form strong hydrophobic interactions. The unsubstituted guanidine moiety of compound (**104**) is oriented differently to the guanidine moiety of the aromatic analogues. This allows (**104**) to form additional hydrogen bonds (**Fig. 15**). The aromatic compounds (**106,110-112,125**) are unable to take up this position due to steric hindrance. If the guanidine moiety of compound (**104**) is positioned as for the aromatic guanidines, the resulting ligand/receptor interactions are weaker. For the guanidinylethyl series of compounds, (**104,106,110-112,125**), both K_D values and interaction energies would predict a trend in binding affinity opposite to that observed in the pharmacological assays (**Table 18**).

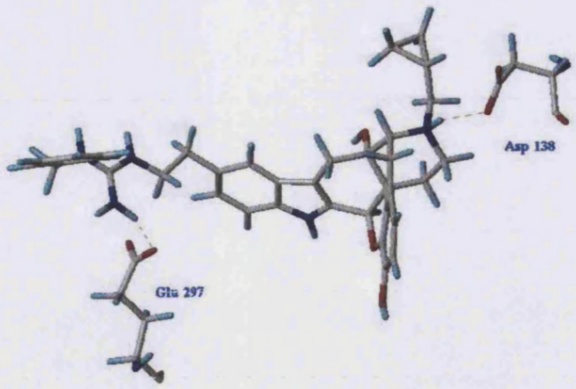


Fig. 14 Ionic interactions and H-bonds between benzyl guanidine (**106**) and the κ-receptor

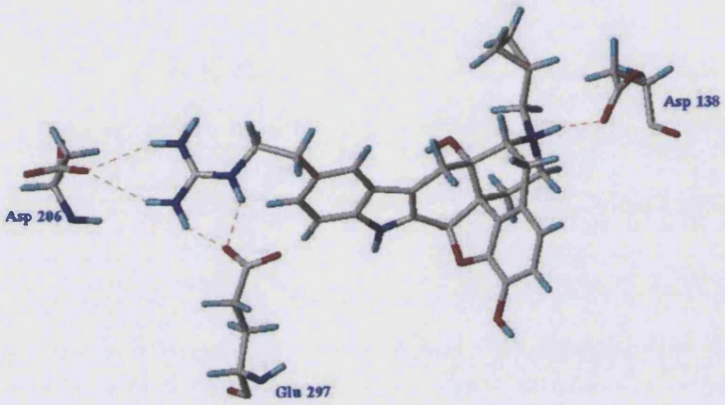


Fig. 15 Ionic interactions and H-bonds between the unsubstituted guanidine (**104**) and the κ-receptor

3.3.9 GUANIDINYL DERIVATIVES (105,107)

The best docking position found for the benzyl and *p*-chlorobenzyl guanidinyl derivatives (**105**,**107**) allowed the formation of a weak ionic interaction between the protonated nitrogen and the carboxylic group of Glu 297. Hydrophobic interactions were seen with residues Leu 309, Ile 290, Phe 314 and Phe 293 (**Table 19**).

Compound	Residue	Interaction	Residue	Distance (Å)	Angle (°)	Interaction
105	Ile 290, Phe 293, Leu 309, Phe 314	Hydrophobic	Glu 297	1.77	122.4	Weak salt bridge
107	Ile 290, Phe 293, Leu 309, Phe 314	Hydrophobic	Glu 297	1.77	122.4	Weak salt bridge

Table 19 Interactions between guanidinyl derivatives (**105**,**107**) and the κ-receptor

Compound	E-Total[a] (kcal/mol)	E-Receptor[b] (kcal/mol)	E-Ligand[c] (kcal/mol)	E-Interaction[d] (kcal/mol)	K_D[e]	K_i[f] (nM)
105	-195.7	-278.0	155.7	-73.4	ND	0.86
107	-198.2	-278.0	155.3	-75.4	ND	0.66

[a]Total energy of the receptor/ligand complex, [b]Energy of the receptor without the ligand, [c]Energy of the minimised ligand, [d](E-Total) – (E-receptor) – (E-Ligand), [e]results from scoring package "SCORE", [f]*cf.* section 4

Table 20 Interaction energy, K_D and K_i values for guanidinyl derivatives (**105**,**107**)

The salt bridge that is able to form between the protonated 5'-side chain and the receptor is slightly weaker than the average ionic interaction, since the bond angle is less than ideal (**Fig. 16**). Nevertheless, this interaction is sufficient to ensure that guanidinyl derivatives (**105**,**107**) show good affinity for the κ-opioid receptor. The aromatic portion of the side chain lies within a hydrophobic pocket formed by residues Phe 293, Ile 290, Leu 309 and Phe 314. π-Stacking interactions, with the aromatic ring of the ligands lying between Phe 314 and Phe 293 (as seen for the aromatic amides), are particularly strong and would therefore enhance ligand affinity. K_i values show little change upon substitution of the aromatic ring (**Table 20**). Once docked into the receptor, the aromatic portion of the ligand lies near, but does not extend beyond, the edge of the receptor. At the moment it is uncertain if there would be other residues in this vicinity (for example from another opioid receptor) with which interactions could take place. The interaction energies for the two receptor/ligand complexes are not vastly different, however they would predict K_i values less similar than those found during the pharmacological assays.

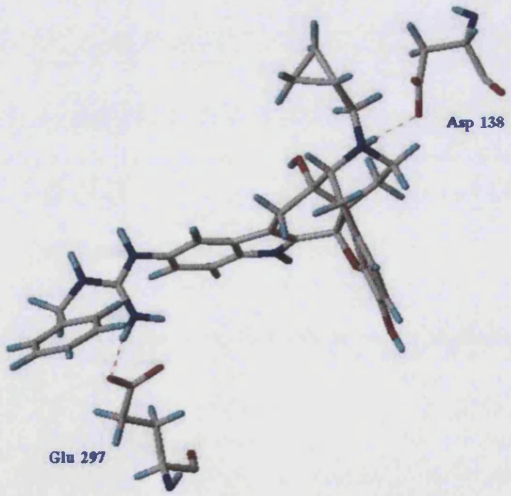


Fig. 16 Ionic interactions and H-bonds between benzyl guanidine (**105**) and the κ-receptor

3.3.10 norBNI (40)

The expected salt bridge could be seen between the second protonated N(17)H and Glu 297. Lipophilic interactions were seen with residues Val 205, Ile 208, Phe 214, Phe 293, Leu 309, Tyr 312 and Phe 314 (**Table 21**).

Compound	Residue	Interaction	Residue	Distance (Å)	Angle (°)	Interaction
40	Val 205, Ile 208, Phe 214, Phe 293, Leu 309, Tyr 312, Phe 314	Hydrophobic	Glu 297	2.16	171.7	Salt bridge

Table 21 Interactions between norBNI (**40**) and the κ-receptor

Compound	E-Total[a] (kcal/mol)	E-Receptor[b] (kcal/mol)	E-Ligand[c] (kcal/mol)	E-Interaction[d] (kcal/mol)	K_D[e]	K_i[f] (nM)
107	-87.5	-280.0	269.6	-77.1	10.00	0.20

[a]Total energy of the receptor/ligand complex, [b]Energy of the receptor without the ligand, [c]Energy of the minimised ligand, [d](E-Total) – (E-receptor) – (E-Ligand), [e]results from scoring package "SCORE", [f]*cf.* section 4

Table 22 Interaction energy, K_D and K_i values for norBNI (**40**)

In agreement with previous findings, norBNI (**40**), is able to form ionic interactions between N(17)H and N(17')H and residues Asp 138 and Glu 297[77] (**Table 22**). Interestingly, the second pharmacophore was positioned such that hydrophobic interactions occurred with both of the hydrophobic pockets identified in section 3.2.7. The second cyclopropylmethyl group was found to interact with residues Phe 293, Phe 314 and Leu 309 (TM 6 and TM7), while the phenolic A ring and piperidine of the address pharmacophore showed hydrophobic interactions with Val 205, Ile 208, Phe 214 and Tyr 312 (mainly in EL 2) (**Fig. 17**).

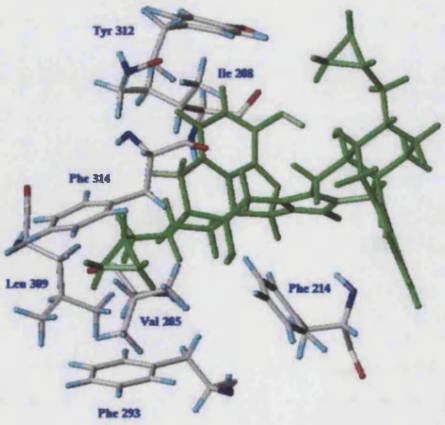
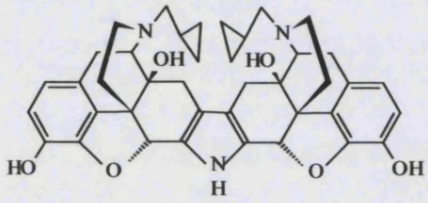


Fig. 17 Hydrophobic interactions between norBNI (**40**) and the κ-receptor

NorBNI (**40**), a bivalent κ-selective antagonist, was of particular interest to us due to its relatively rigid structure. It was once believed that norBNI might bind to two receptors, causing dimerisation. This has however been shown to be unlikely, since an isomeric form of norBNI meso-norBNI (**136**),[51] in which one of the pharmacophores was substituted with the enantiomeric (inactive) pharmacophore, also displays potent kappa-selective antagonist activity. We were able to dock meso-norBNI (**136**) into our receptor model, showing that the second pharmacophore would again form hydrophobic contacts with the residues in these two pockets, and kappa selectivity would therefore be expected in spite of the inactive pharmacophore (**Fig. 18**).



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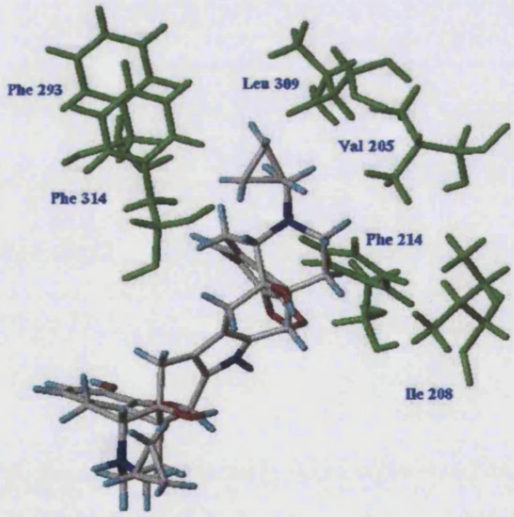
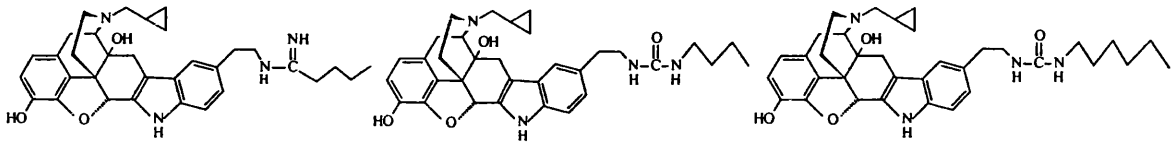


Fig. 18

3.4 COMPARISON OF DOCKING AT DIFFERENT OPIOID RECEPTORS

After considering the interaction of these compounds with the κ-receptor (*ie.* looking at affinity), an investigation was made into selectivity by comparing these interactions with interactions at the μ- and δ-receptors.

The *n*-butyl amidine (**70**)[74] was selected as an example of a compound showing greater affinity for the κ-opioid receptor than for either the μ- or δ-receptors. The hexylurea (**87**) showed slightly greater affinity for the δ-opioid receptor than for the κ-receptor, while urea (**86**), having a butyl side chain, showed approximately equal affinity for the μ- and κ-opioid receptors.



70 **86** **87**

The results of preliminary docking studies conducted with these ligands are shown below. Interaction energies are quoted as a form of comparison, however, these values may be misleading due to the differences between the individual receptors. Greater insight can be gained by examining the receptor/ligand complexes for ionic interactions, hydrogen bonds and hydrophobic interactions.

3.4.1 COMPARISON OF BINDING TO κ- AND δ-RECEPTORS

Amidine (**70**) was docked into the κ-receptor as described in sections 3.2.7 and 3.3.3 (**Fig. 19**). Ionic interactions were seen between N(17)H and Asp 138 and between the protonated 5'-side chain and Glu 297. Additional hydrogen bonds were able to form between the pyrrole NH group and Glu 209, the phenolic hydroxyl group and His 291 and between the side chain NH group and Glu 297. Hydrophobic interactions were made with the lipophilic residues Val 134, Ile 135, Tyr 139, Met 142, Val 205, Ile 208, Leu 212, Phe 214, Trp 287, Ile 290, Ile 294, Leu 309, Ile 316 and Leu 318 (**Table 23**). The interaction energy found for amidine (**70**) with the κ-opioid receptor is –70.4 kcal/mol.

Compound (**70**) was then docked into the δ-receptor,[189] and manually manipulated and minimised until the best docking position was found (**Fig. 20**). A salt bridge could be seen between N(17)H and Asp 128, while C(4)O formed a hydrogen bond to Trp 274. The hydrophobic residues Val 124, Tyr 129, Val 196, Leu 200, Lys 214, Phe 218, Phe 222, Ile 277, Trp 274, Val 281, Ile 282 and Thr 285 were also able to interact with amidine (**70**). No ionic interactions or hydrogen bonds could be seen between the 5'-side chain and the δ-opioid receptor (**Table 23**). The interaction energy found for amidine (**70**) with the δ-receptor is –46.7 kcal/mol.

Compound	Ionic interactions	Residue	H-bonds	Residue	Hydrophobic interactions
70 κ-receptor	N(17)H 5'-C=NH_2^+	Asp 138 Glu 297	C(3)OH pyrrole NH 5'-NH	His 291 Glu 209 Glu 297	Val 134, Ile 135, Tyr 139, Met 142, Val 205, Ile 208, Leu 212, Phe 214, Trp 287, Ile 290, Ile 294, Leu 309, Ile 316, Leu 318
70 δ-receptor	N(17)H	Asp 128	C(4)O	Trp 274	Val 124, Tyr 129, Val 196, Leu 200, Lys 214, Phe 218, Phe 222, Ile 277, Trp 274, Val 281, Ile 282, Thr 285

Table 23 Interactions between amidine (**70**) and the κ- and δ-receptors

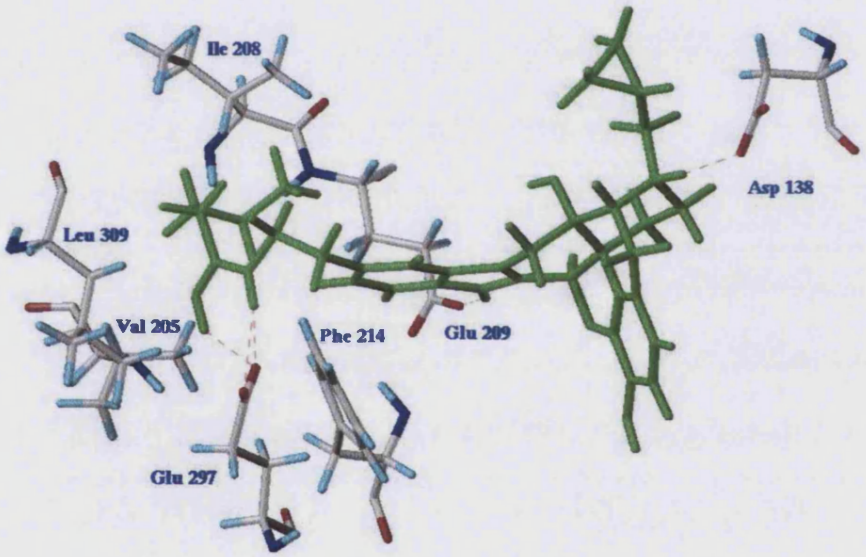


Fig. 19 Ionic interactions and H-bonds between amidine (**70**) and the κ-receptor

As can be seen from the above table, the ionic interactions and hydrogen bonding between amidine (**70**) and the κ-receptor are far superior to those that are able to form between (**70**) and the δ-receptor. This would result in greater affinity for the κ-opioid receptor. A comparison of the interaction energies further confirms this finding.

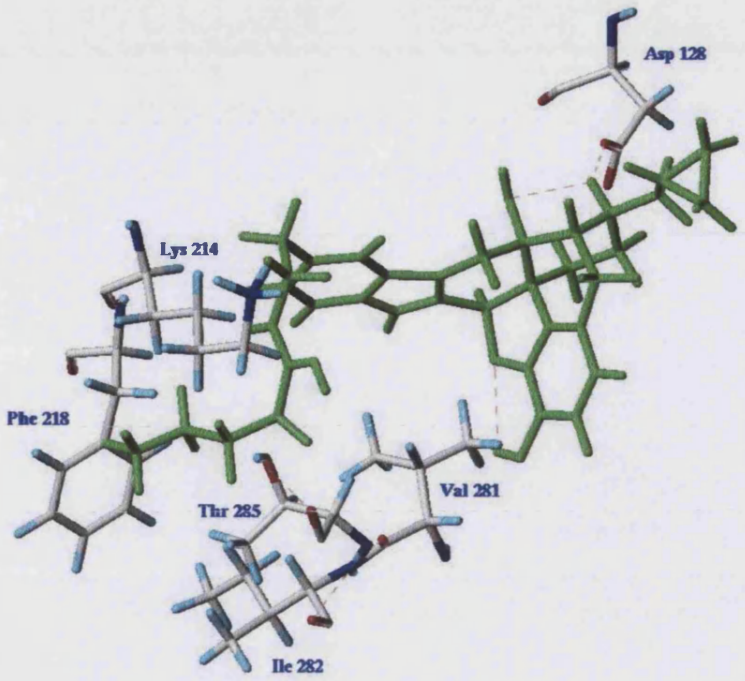


Fig. 20 Ionic interactions and H-bonds between amidine (**70**) and the δ-receptor

The urea derivative (**87**) was docked into the κ-receptor as described in sections 3.2.7 and 3.3.6 (**Fig. 21**). Ionic interactions were seen between N(17)H and Asp 138. Additionally hydrogen bonds could be seen between the pyrrole NH group and Glu 209 and between the phenolic hydroxyl group and His 291. Lipophilic interactions were made with the hydrophobic residues Val 134, Ile 135, Tyr 139, Met 142, Leu 212, Trp 287, Ile 290, Phe 293, Ile 294, Leu 309, Phe 314, Ile 316 and Leu 318 (**Table 24**). The interaction energy for urea (**87**) and the κ-receptor is –62.0 kcal/mol.

The docking position of urea (**87**) within the δ-receptor,[189] was improved by manual manipulation and minimisation, until the optimum position was found (**Fig. 22**). A salt bridge could be seen between N(17)H and Asp 128. Hydrogen bonds were formed between C(4)O and Trp 274, between the carbonyl of the urea moiety and Lys 214 and between the second NH group of the urea and Asp 210. The lipophilic residues Val 124, Tyr 129, Val 196, Leu 200, Lys 214, Val 217, Leu 200, Phe 222, Ile 277, Trp 274, Val 281 and Leu 300 were able to form hydrophobic interactions with urea (**87**) (**Table 24**). The interaction energy found for urea (**87**) with the δ-receptor is –70.3 kcal/mol.

Compound	Ionic interactions	Residue	H-bonds	Residue	Hydrophobic interactions
87 κ-receptor	N(17)H	Asp 138	C(3)OH pyrrole NH	His 291 Glu 209	Val 134, Ile 135, Tyr 139, Met 142, Leu 212, Trp 287, Ile 290, Phe 293, Ile 294, Leu 309, Phe 314, Ile 316, Leu 318
87 δ-receptor	N(17)H	Asp 128	C(4)O C=O NHCONH	Trp 274 Lys 214 Asp 210	Val 124, Tyr 129, Val 196, Leu 200, Lys 214, Val 217, Leu 200, Phe 222, Ile 277, Trp 274, Val 281,Leu 300

Table 24 Interactions between urea (**87**) and the κ- and δ-receptors

The interaction energy for urea (**87**) and the κ-opioid receptor is relatively high, although there are no ionic interactions between the 5'-side chain and the receptor. This could be attributed to strong hydrophobic interactions, which would increase the affinity of the ligand for the receptor. However, when urea (**87**) is docked into the δ-opioid receptor, the 5'-side chain is able to form two hydrogen bonds to residues within the δ-receptor active site. Ionic interactions, hydrogen bonds and hydrophobic interactions with the naltrindole portion of the molecule further contribute to the increased affinity shown by (**87**) for the δ-receptor. The interaction energy for (**87**) in the δ-receptor is also lower than that for the κ-receptor, indicating greater affinity. The interaction of the carbonyl group of urea (**87**) with Lys 214 is particularly interesting since a similar interaction has been proposed for δ-selective peptides.[190]

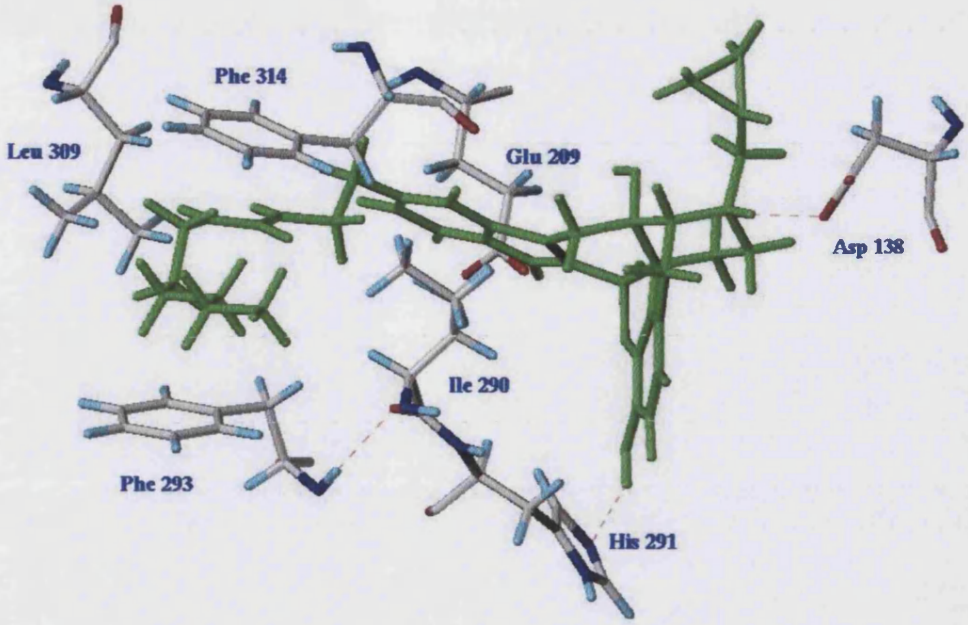


Fig. 21 Ionic interactions and H-bonds between urea **(87)** and the κ-receptor

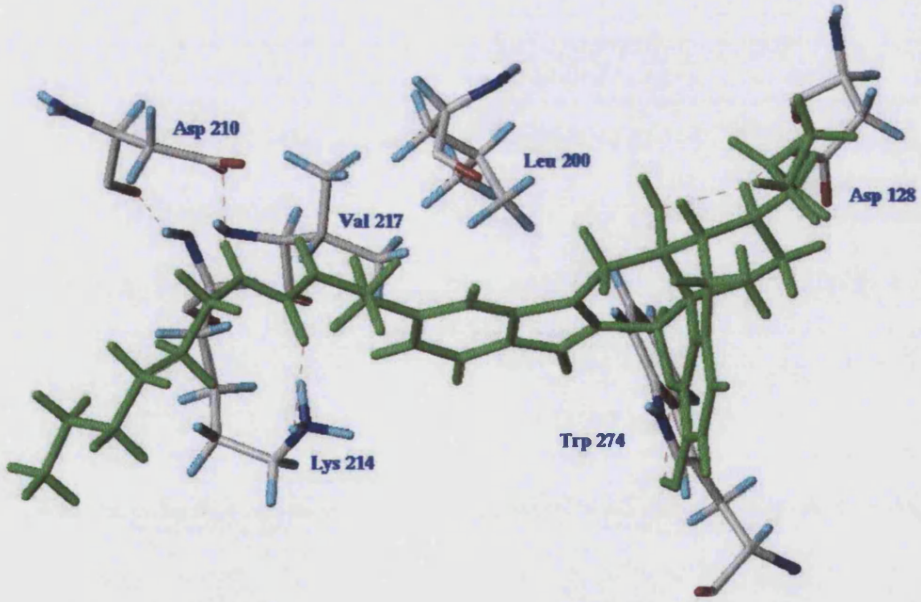


Fig. 22 Ionic interactions and H-bonds between urea **(87)** and the δ-receptor

3.4.2 COMPARISON OF BINDING TO κ- AND μ-RECEPTORS

The docking position of amidine (**70**) is described in section 3.4.1 (**Fig. 19**). A summary of the interactions described in that section can be found in **Table 25**. The interaction energy found for amidine (**70**) with the κ-opioid receptor is –70.4 kcal/mol.

The butyl amidine (**70**) was docked into the μ-receptor,[189] and manually manipulated and minimised until the best docking position was found (**Fig. 23**). An ionic interaction was able to form between N(17)H and the acidic Asp 147 residue. In addition, this residue was able to form a hydrogen bond with the C(14)OH group. Lipophilic interactions could be seen between the ligand and residues Tyr 148, Met 151, Val 236, Ile 296, Val 300, Ile 301 and Phe 320 (**Table 25**). The interaction energy found for amidine (**70**) in the μ-receptor is –33.3 kcal/mol.

Compound	Ionic interactions	Residue	H-bonds	Residue	Hydrophobic interactions
70 κ-receptor	N(17)H 5'-C=NH_2^+	Asp 138 Glu 297	C(3)OH pyrrole NH 5'-NH	His 291 Glu 209 Glu 297	Val 134, Ile 135, Tyr 139, Met 142, Val 205, Ile 208, Leu 212, Phe 214, Trp 287, Ile 290, Ile 294, Leu 309, Ile 316, Leu 318
70 μ-receptor	N(17)H	Asp 147	C(14)OH	Asp 147	Tyr 148, Met 151, Val 236, Ile 296, Val 300, Ile 301, Phe 320

Table 25 Interactions between amidine (**70**) and the κ- and μ-receptors

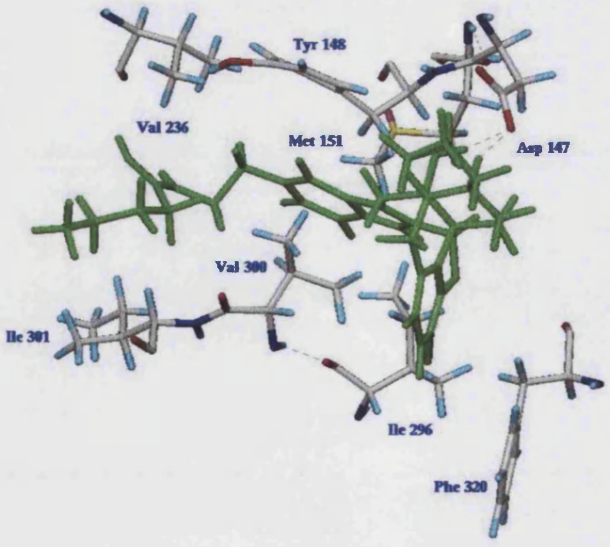


Fig. 23 Ionic interactions and H-bonds between urea (**87**) and the μ-receptor

From **Table 25**, it can clearly be seen that amidine (**70**) is able to form a greater number of ionic interactions, hydrogen bonds and hydrophobic interactions with the κ-receptor than with the μ-receptor. This results in the affinity of amidine (**70**) for the κ-receptor being significantly higher than the affinity for the μ-receptor. The interaction energies for compound (**70**) with each of the receptors clearly reflect these differences.

The *n*-butylurea (**86**) was docked into the κ-receptor as described in sections 3.2.7 and 3.3.6 (**Fig. 24**). The docking position allowed the formation of an ionic interaction between N(17)H and Asp 138, as well as hydrogen bonds between the pyrrole NH group and Glu 209 and between the phenolic hydroxyl group and His 291. Lipophilic interactions were made with the hydrophobic residues Val 134, Ile 135, Tyr 139, Met 142, Leu 212, Trp 287, Ile 290, Phe 293, Ile 294, Phe 314, Ile 316 and Leu 318 (**Table 26**). The interaction energy for urea (**86**) and the κ-receptor is –59.2 kcal/mol.

Urea (**86**) was placed in such a position within the μ-opioid receptor, so as to allow the formation of a salt bridge interaction between N(17)H and Asp 147.[189] The position was then improved by manual manipulation and minimisation, until the optimum position was found (**Fig. 25**). Hydrogen bonds could be seen between the C(14)OH group and Asp 147, and between the carbonyl group of the urea and His 297. The hydrophobic residues Tyr 148, Val 236, Phe 237, Ile 296, Val 300, Ile 301 and Phe 320, were able to form lipophilic interactions with urea (**86**) (**Table 26**). The interaction energy found for urea (**87**) with the μ-receptor is -40.2 kcal/mol.

Compound	Ionic interactions	Residue	H-bonds	Residue	Hydrophobic interactions
86 κ-receptor	N(17)H	Asp 138	C(3)OH pyrrole NH	His 291 Glu 209	Val 134, Ile 135, Tyr 139, Met 142, Leu 212, Trp 287, Ile 290, Phe 293, Ile 294, Leu 309, Phe 314, Ile 316, Leu 318
86 μ-receptor	N(17)H	Asp 147	C(14)OH C=O	Asp 147 His 297	Tyr 148, Val 236, Phe 237, Ile 296, Val 300, Ile 301, Phe 320

Table 26 Interactions between urea (**86**) and the κ- and μ-receptors

The 5'-side chain of urea (**86**) is unable to form any ionic interactions or hydrogen bonds with the κ-opioid receptor. With the μ-receptor, however, the carbonyl group of the urea moiety is able to interact with the basic His 297 residue. This would strengthen the affinity of the ligand for the receptor. The naltrindole portion of urea (**86**) is however able to form stronger interactions with the κ-receptor. In practice, the affinity of urea (**86**) for the κ- and μ-receptors is

approximately equal (*cf.* 6.33 nM and 4.80 nM respectively). This result could not have been predicted by looking at the interaction energies of the two receptor/ligand complexes.

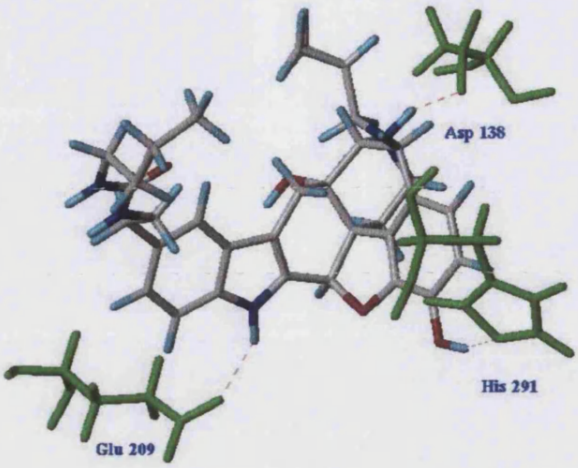


Fig. 24 Ionic interactions and H-bonds between urea (**86**) and the κ-receptor

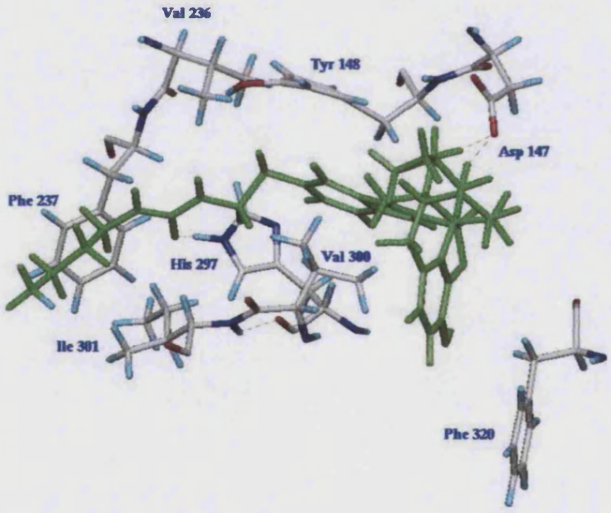


Fig. 25 Ionic interactions and H-bonds between urea (**86**) and the μ-receptor

3.5 CONCLUSIONS AND FUTURE WORK

Docking of the various ligands to the model of the κ-receptor resulted in a complex that utilised residues, within the receptor, that had previously been shown to be of importance (mutation and chimera studies). It proved impossible to find a quantitative relationship that held for all the structures docked. This can, in part, be attributed to the shortcomings of scoring functions as discussed in section 3.1. The further development of such a model would therefore be aided by the inclusion of factors such as entropy and solvation. The κ-affinities of the compounds

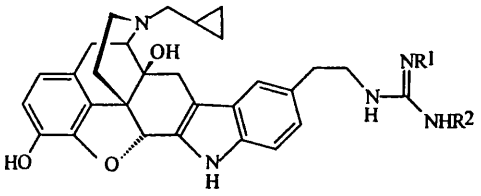
modelled in this study were not vastly dissimilar, making consistent prediction by the model more difficult.

It was, however, possible to derive qualitative descriptions of the interactions involved, and in this way, to provide a degree of post-hoc rationalisation of the pharmacological data. The hope was to identify common trends in series showing both high and low selectivity. Additionally, potential structures were sought, which would show good interactions with the modelled receptor, in the hope that synthesis and subsequent pharmacological testing of these compounds might lead to novel, potent and selective ligands.

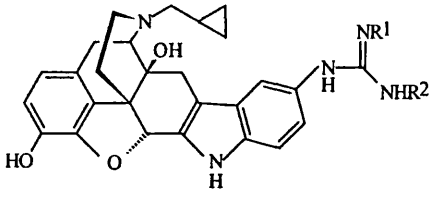
For all the compounds docked, the presence of a protonated nitrogen group in the 5'-side chain, which could be suitably positioned for ionic interaction with the acidic Glu 297 residue, ensured good affinity for the κ-opioid receptor. This caused a further increase in selectivity by decreasing affinity at the μ- and δ-receptors. Since the presence of a carbonyl group in the 5'-side chain increased affinity for the μ- and δ-receptors, this group should be omitted from the 5'-side chain of potential κ-selective ligands. As the κ-selectivity studies were only conducted on the above three ligands, further conclusions, such as the influence of steric bulk, cannot be discussed at this stage.

By examining the docking of ligands (**59-63,70-74,76,82-87,98,104-107,110-112,125,130-135**) within the κ-opioid receptor, further modifications that might increase ligand affinity and/or selectivity were identified.

The FlexX generated docking positions highlighted two hydrophobic pockets with which the aliphatic portion of the 5'-side chain could interact (*cf.* section 3.2.6). Interestingly, norBNI (**40**), a highly potent and selective κ-antagonist, was able to interact with both of these hydrophobic pockets (*cf.* section 3.3.10). It was reasoned therefore that the addition of a second aliphatic moiety to our basic 5'-side chain, could increase affinity for the κ-receptor and, due to increased steric bulk, possibly also decrease affinity for the μ- and δ-receptors. Compounds (**113-117,** section 2.6) were synthesised in order to test this hypothesis. The pharmacological results are however still outstanding.

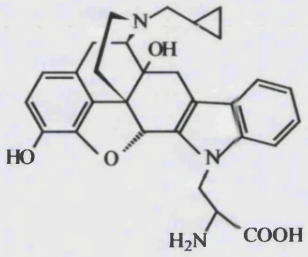


113 $R^1 = R^2 = (CH_2)_3CH_3$



114 $R^1 = R^2 = (CH_2)_3CH_3$
115 $R^1 = R^2 = (CH_2)_2CH_3$
116 $R^1 = (CH_2)_2CH_3$, R^2 = cyclopropylmethyl
117 $R^1 = CH_2C_6H_5$, R^2 = cyclopropylmethyl

The indole NH group is able to form a hydrogen bond with Glu 209. Replacement of the NH by an NH_2^+ group,would allow a stronger ionic interaction to form. A basic Lys 227 residue, which would be able to form an ionic interaction with a negatively charged moiety, is also in close proximity to the indole. A compound such as (**137**) would be able to interact with both of these residues forming strong ionic interactions (**Fig. 26**) and enhancing the affinity of the ligand for the receptor. Compounds of this nature have yet to be made and tested.



137

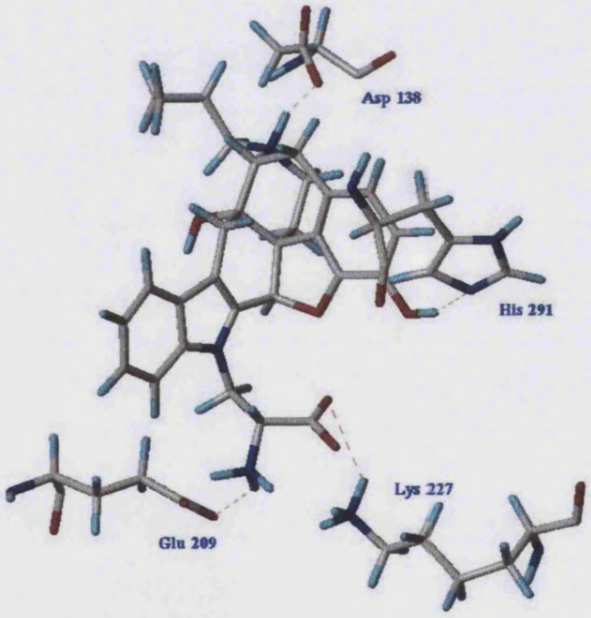


Fig. 26

4. PHARMACOLOGY

4.1 INTRODUCTION

In the early years of opioid research, the pharmacological activity of new opioid ligands could only be evaluated by means of *in vivo* investigations. These techniques involved assessing the response of test animals to nociceptive stimuli, before and after treatment with the test ligand. The potency of different opioid drugs could be quantitatively compared by measuring the response time, or number of responses over a given period of time. The antagonist ability of a compound could be measured in terms of its ability to reverse the antinociceptive effects of a known opioid agonist, in an animal subjected to nociceptive stimuli.

The potential for misinterpretation of results exists – of particular significance, the morphine antagonist, nalorphine, was originally shown to be inactive as an agonist in animal nociceptive tests,[191] but later displayed analgesic properties in humans.[31] More recently however, antinociceptive tests showing greater sensitivity have been developed (eg. abdominal stretch). These tests are able to provide information on κ- and δ-, as well as μ-activity.

Recognizing the presence of opioid receptors in several peripheral tissues, Kosterlitz *et al.*[192] developed the guinea-pig *ileum* (GPI) and mouse *vas deferens* (MVD) isolated tissue assays during the 1960's. These were assays identifying the agonist or antagonist properties of a test compound. Secondary factors, such as drug distribution, metabolism and excretion, which complicate *in vivo* investigations, are less likely to play a role in isolated tissue assays. The application of *in vitro* assays has allowed greater insight into the structure-activity relationships found in opioid ligands.

The use of radioligand binding techniques has greatly increased, particularly since the identification and subsequent purification of homogenous populations of receptor types. At low concentrations, a drug will selectively bind to the site for which it has the highest affinity.[193] Hence, by introducing a radioactive marker into a selective ligand, the amount of ligand bound to a specific tissue sample can be measured, and the relative affinity of the test compound for each receptor type can be determined.

Following the cloning of opioid receptors in the 1990's,[6] assays have been developed which measure the stimulation of individual cloned receptors. Single human opioid receptor types have been transfected into Chinese Hamster Ovary cells.[194] Binding of an agonist to these cloned receptors causes stimulation of [^{35}S] GTPγS binding. The extent of stimulation of [^{35}S] GTPγS binding can be measured by radio-emission. Functional assays of the above type are now replacing the MVD and GPI assays.

4.2. *IN VITRO* ASSAYS

4.2.1 BINDING ASSAYS

Radioligand binding studies are used to determine ligand binding affinity, and hence selectivity, of various ligands for the opioid receptor types. Tritium, and less frequently [^{125}I], have been incorporated into the selective agonists DAMGO (**11**) (μ), DPDPE (**13**) (δ) and U69593 (**16**) (κ), and displacement of these radioligands from receptors by a test compound gives a measure of ligand affinity.

The ability of unlabeled test compounds to compete for binding sites with receptor selective radiolabeled ligands, gives an indication of the affinity of the unlabeled compound for the specific receptor type. The concentration of competitor necessary to lower the binding of the radioligand by 50% (IC_{50}) can be determined. This figure is used as an indication of the affinity of the test compound for the receptor. The IC_{50} value is related to the dissociation constant for the inhibitor (K_i) by equation (1):[195]

$$K_i = IC_{50}/(1 + ([D]/K_D)) \qquad (1)$$

where,

K_I = dissociation constant of inhibitor

IC_{50} = concentration of inhibitor lowering binding by 50%

[D] = concentration of radioligand

K_D = dissociation constant of radioligand

Since the IC_{50} value is a function of the concentration of radioligand, care must be taken to ensure that identical assay conditions are used when comparing data. K_i is however independent of [D], and is thus a much more reliable comparison of the affinity of different test ligands.

4.2.2 FUNCTIONAL ASSAYS

Until recently, the tissue preparations most commonly used in *in vitro* assays were the myenteric plexus-longitudinal-muscle preparation from the isolated *ileum* of the guinea pig (GPI),[196] which contains mainly μ- and κ-receptors, and the mouse *vas deferens* (MVD),[192] which contains all three receptor types. The effect of an opioid agonist is to dose-dependently inhibit the electrically induced muscle contraction of the sample tissue. Antagonist action can be measured by the extent to which contraction is re-established on administration of the test drug to a tissue pre-treated with a standard selective agonist.

In the mid 1990's Traynor and Nahorski[197] showed the binding of the GTP analogue, guanosine-5'-*O*-(3-[^{35}S]thio)triphosphate ([^{35}S] GTPγS), to cell membranes of the human neuroblastoma SH-SY5Y cell line, to be a concentration dependent, opioid receptor-mediated process. Assays have since been developed, making use of cloned single human opioid receptor types, which have been transfected into Chinese Hamster Ovary cells.[194] The opioid agonist-mediated stimulation of [^{35}S] GTPγS binding in the cell membranes of these cells thus provides a functional measure of agonist occupation of opioid receptors and offers a simple method for the determination of efficacy and intrinsic activity of opioid agonists. The use of [^{35}S] GTPγS assays has now superseded the use of isolated tissue assays. For antagonists, the equilibrium dissociation constant, K_e, is calculated.

4.3 *IN VIVO* ASSAYS

I*n vivo* assays are primarily based upon the response of rodents to various nociceptive stimuli such as temperature, (eg. mouse hot plate, rat tail flick and mouse warm water tail withdrawal tests), pressure, (rat tail pressure and mouse tail pinch tests), chemical irritants (mouse abdominal stretch test) and electric shock.

Thermal assay procedures are widely used, and are useful in assessing the efficacy of μ-agonists and antagonists by utilizing different water temperatures. At higher temperatures (55 °C as opposed to 50 °C) the actions of a partial agonist may be ineffective in overcoming the nociceptive stimulus, thereby allowing its antagonist actions to become demonstrable. A competitive antagonist would shift the dose-response curve of a known agonist to the right. The dose-response curve obtained in the presence of a non-competitive (irreversible) antagonist, would be flatter as well as shifted to the right. This implies an increase in the dose of the agonist required to get the same effect, as well as a decrease in the maximum effect attainable.[198]

Intraperitoneal injection of chemical irritants such as phenylquinone, acetylcholine and acetic acid cause an abdominal stretch response in rodents, which can be blocked by various analgesic agents. The average number of abdominal stretches (arching of the back, pelvic rotation or hind limb rotation) in a group of rodents, pretreated with a test compound, can be counted and compared to the average number of stretches in an untreated control group. In a study by Tyers and co-workers, in which various *in vivo* assays were compared, the mouse abdominal stretch assay was able to detect the greatest range of agonists with the greatest sensitivity.[200] Agonists at κ- and δ-receptors have been shown to be consistently active only in the anti-stretch assays,[201] although κ-agonists of high efficacy are active in thermal models. Additionally, the selectivity of antagonist actions of a test compound can be determined by use of selective prototype agonists.

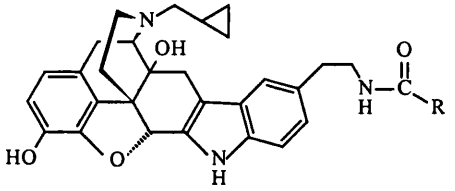
At the time of submission, *in vivo* test results for our compounds were still outstanding.

4.4 RESULTS

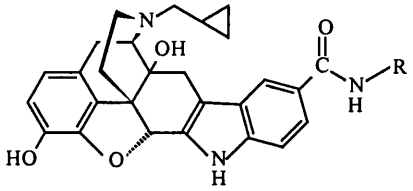
In each case the standard κ-selective opioid antagonist norBNI (**40**)[62], tested under similar conditions to our compounds, has been included in the tables for comparison.

4.4.1 AMIDE SUBSTITUENTS

Compounds **59–63** (**Table 27**) were prepared as described in section 2.1 and submitted for pharmacological evaluation as the hydrochloride salts. These were compared to compounds (**130-132**), prepared by Jales[74] and submitted for pharmacological evaluation as the hydrochloride salts.



A



B

Compound	Structure	R	BU Number
130*	A	CH_2CH_3	98022
131*	A	$(CH_2)_2CH_3$	98023
132*	A	$(CH_2)_3CH_3$	98024
59	B	$(CH_2)_6CH_3$	99028
60	B	$CH_2C_6H_5$	99036
61	B	$(CH_2)_2C_6H_5$	99037
62	B	$(CH_2)_4C_6H_5$	20001
63	B	$CH_2C_6H_4OCH_3(p)$	20021

* from reference 74

Table 27 Amide substituted compounds submitted for pharmacological testing

The binding affinities of the compounds were measured in cloned human opioid receptors (**Table 28**). Data provided by NIDA Opiate Treatment Drug Discovery Program (under contract to SRI)

	K_i (nM) ± SEM					
Compound	μ [^{3}H]-DAMGO	δ [^{3}H]-DPDPE	κ [^{3}H]U69,593	n	μ/κ	δ/κ
130*	22.97 ± 11.10	3.12 ± 0.50	1.57 ± 0.80	2	15	2
131*	30.97 ± 0.10	3.70 ± 0.34	0.85 ± 0.40	2	36	4
132*	31.59 ± 0.59	7.67 ± 0.64	0.68 ± 0.30	2	46	11
59	61.91 ± 6.03	70.15 ± 33.93	21.89 ± 7.11	2	3	3
60	43.95 ± 8.27	11.19 ± 2.86	10.33 ± 0.66	2	4	1
61	23.53 ± 7.32	3.53 ± 1.24	2.21 ± 0.35	2	11	2
62	37.05 ± 9.45	59.45 ± 4.82	6.18 ± 0.47	2	6	10
63	4.64 ± 0.58	7.41 ± 0.59	2.11 ± 1.04	2	2	4
40, norBNI	21.0 ± 5.0	5.7 ± 0.9	0.20 ± 0.05	2	105	28

* from reference 74

Table 28 Binding affinities measured in cloned human opioid receptors

[^{35}S] GTPγS assays were performed in cloned human opioid receptors (**Table 29**). Data provided by NIDA Opiate Treatment Drug Discovery Program (under contract to SRI)

	K_e (nM) ± SEM					
Compound	**μ-CHO membranes DAMGO**	**δ-CHO membranes DPDPE**	**κ-CHO membranes U69,593**	**n**	**μ/κ**	**δ/κ**
130*	4.94 ± 1.08	0.38 ± 0.05	0.48 ± 0.27	4	10	1
131*	3.17 ± 0.34	0.30 ± 0.10	0.35 ± 0.23	4	9	1
132*	3.37 ± 1.22	0.23 ± 0.04	0.46 ± 0.14	5	7	0.5
59	6.86 ± 0.93	6.95 ± 0.86	0.29 ± 0.08	5	24	24
60	4.40 ± 0.74	2.99 ± 0.22	0.73 ± 0.04	5	6	4
61	2.70 ± 0.31	1.21 ± 0.05	0.17 ± 0.03	6	16	7
62	2.78 ± 0.21	5.15 ± 0.25	0.26 ± 0.02	5	10	20
63	0.94 ± 0.12	6.20 ± 0.49	0.28 ± 0.04	8	3	22
40, norBNI	18.9 ± 1.8	4.42 ± 0.38	0.04 ± 0.004		484	113

* from reference 74

Table 29 [^{35}S] GTPγS assays performed in cloned human opioid receptors

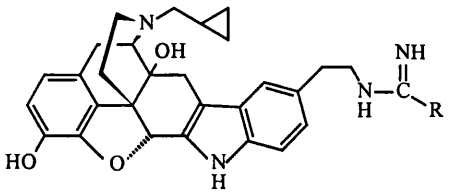
Of the more recently synthesised amides, (**59**) is most closely related to the original amides prepared by Jales.[74] The heptyl side chain provides a total chain length of 9 atoms, only one more than for amide (**132**). In binding, (**59**) was of much lower affinity than (**130-132**), and displayed little selectivity for the κ-opioid receptor.

The apparent link between κ-affinity and lipophilicity was further explored with the synthesis of amides (**60-63**), each having an aryl substituent. In comparison to the alkyl amide (**59**), the aryl amides (**60-63**) show higher affinity for all three receptor types. A clear trend can be seen within this series, with the phenethyl amide (**61**) having higher affinity at each receptor than either its shorter chain benzyl analogue (**60**) or the longer chain phenylbutyl amide (**62**). The introduction of a *p*-methoxy group into (**60**) to give (**63**) resulted in an increase in affinity at each receptor type, but particularly at μ (10 fold) and κ (5 fold).

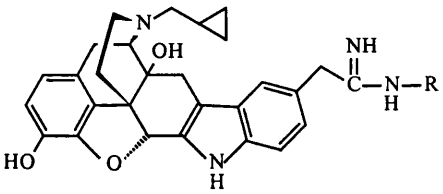
In keeping with the results of the binding assays, in the GTPγS assays the phenethyl amide (**61**) again showed somewhat higher antagonist potency at either receptor than either (**60**) or (**62**). (**63**), the *p*-methoxy analogue of (**60**), again displayed higher antagonist potency at μ (5 fold) and κ (2-3 fold), but reduced potency at δ (2 fold). The K_e of an antagonist determined in the functional assays should approximate to the K_i determined in binding assays. For the amides, the K_e was consistently 5-10 fold lower (more potent) than the K_i would have predicted at μ- and δ-receptors and 10-100 fold lower at κ-receptors. As a result, the compounds appear somewhat more potent and selective in the functional assays than in binding assays. This was particularly notable for the heptylamide (**59**), which displayed a 100 fold increase at κ, but only a 10 fold increase at μ and δ, resulting in reasonable κ-antagonist activity and selectivity.

4.4.2 AMIDINE SUBSTITUENTS

Compound (**71**) was prepared as described in section 3.2 and submitted for pharmacological evaluation as the hydrochloride salt. (**72-74**) Were prepared as described in section 2.2 and submitted for pharmacological evaluation as the hydrobromide salts (**Table 30**). These were compared to compounds (**133-135,70**), prepared by Jales[74] and submitted for pharmacological evaluation as the hydrochloride salts.



A



B

Compound	Structure	R	BU Number
133*	A	CH_3	98018
134*	A	CH_2CH_3	98019
135*	A	$(CH_2)_2CH_3$	98020
70*	A	$(CH_2)_3CH_3$	98021
71	A	$CH_2CH(CH_3)_2$	99021
72	B	$(CH_2)_2CH_3$	99029
73	B	$(CH_2)_4CH_3$	99030
74	B	$(CH_2)_6CH_3$	99031

*from reference 74

Table 30 Amidine substituted compounds submitted for pharmacological testing

The binding affinities of the compounds were measured in cloned human opioid receptors (**Table 31**). Data provided by NIDA Opiate Treatment Drug Discovery Program (under contract to SRI)

	K_i (nM) ± SEM					
Compound	μ [^{3}H]-DAMGO	δ [^{3}H]CI-DPDPE	κ [^{3}H]U69,593	n	μ/κ	δ/κ
133*	22.32 ± 2.27	21.38 ± 1.32	0.29 ± 0.10	2	77	74
134*	26.35 ± 0.46	21.76 ± 1.50	0.28 ± 0.10	2	94	78
135*	40.79 ± 6.32	27.56 ± 2.11	0.25 ± 0.10	2	163	110
70*	219.16 ± 84.47	38.22 ± 4.91	0.30 ± 0.20	2	730	127
71	47.40 ± 7.07	20.10 ± 4.29	1.39 ± 0.14	2	34	14
72	13.48 ± 0.54	5.29 ± 0.27	1.60 ± 0.28	2	8	3
73	25.29 ± 4.06	17.02 ± 7.19	1.44 ± 0.04	2	18	12
74	56.62 ± 7.69	7.33 ± 1.13	5.61 ± 0.37	2	10	1
40, norBNI	21.0 ± 5.0	5.7 ± 0.9	0.20 ± 0.05	2	105	28

*from reference 74

Table 31 Binding affinities measured in cloned human opioid receptors

[^{35}S] GTPγS assays were performed in cloned human opioid receptors (**Table 32**). Data provided by NIDA Opiate Treatment Drug Discovery Program (under contract to SRI)

	K_e (nM) ± SEM					
Compound	**μ-CHO membranes DAMGO**	**δ-CHO membranes DPDPE**	**κ-CHO membranes U69,593**	**n**	**μ/κ**	**δ/κ**
133*	3.78 ± 0.68	1.79 ± 0.69	0.21 ± 0.04	6	18	9
134*	4.70 ± 1.34	1.77 ± 0.25	0.24 ± 0.03	4	20	7
135*	4.21 ± 1.61	1.89 ± 0.33	0.18 ± 0.06	4	23	11
70*	5.33 ± 0.63	3.31 ± 0.54	0.17 ± 0.05	5	31	20
71	14.73 ± 0.83	5.23 ± 0.13	0.32 ± 0.02	5	46	16
72	3.19 ± 0.25	4.41 ± 0.79	0.05 ± 0.004	4	64	88
73	5.61 ± 0.28	3.83 ± 0.40	0.21 ± 0.03	5	27	18
74	2.04 ± 0.62	5.83 ± 0.42	0.37 ± 0.06	5	6	16
40, norBNI	18.9 ± 1.8	4.42 ± 0.38	0.04 ± 0.004		484	113

*from reference 74

Table 32 [^{35}S] GTPγS assays performed in cloned human opioid receptors

Jales[74] previously reported on the trend that in the n-alkyl amidine series (**133-135,70**), an increase in κ-selectivity accompanied an increase in the length of the alkyl side chain. This was due to a decrease in affinity at μ- and δ-receptors, rather than an increase in affinity at the κ-receptor. In a related series of amidines, Portoghese and Olmsted[72] showed that introducing a branch into the alkyl chain increased κ-selectivity (*cf.* section 2.2.1). The isobutyl amidine (**71**), a branched analogue of the propyl amidine (**135**), was synthesised to further investigate these findings. Comparison of the K_i values for (**135**) and (**71**), show that while μ- and δ-affinities remain unchanged, the affinity of the branched analogue (**71**) for the κ-receptor is greatly reduced and hence the compound is less κ-selective in binding assays. As seen for the n-alkyl amidine series (**133-135,70**), for the reverse amidine series (**72-74**), an increase in alkyl chain length causes a decrease in μ-affinity. No consistent trend can be established for δ-receptor affinity. The affinities of the propyl (**72**) and pentyl (**73**) reverse amidines for the κ-receptor are approximately equal, with only the heptyl reverse amidine (**74**) showing a decrease in affinity. This could indicate that the heptyl side chain is beginning to exceed the steric capacity of the κ-receptor.

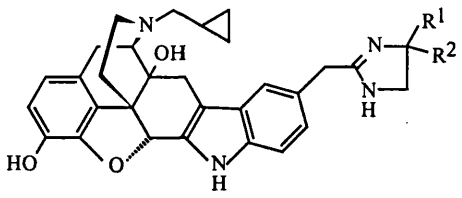
In the functional assay, the trends displayed in the binding assay for the n-alkyl amidines (**133-135,70**) were again repeated. There was a slight decrease in antagonist potency at both μ and δ for the isobutyl amidine (**71**) as compared to the straight chain analogues (**133-135,70**). Antagonist potency at the κ-receptor was however essentially unchanged, resulting in greater κ-

selectivity for (**71**) – a result which agrees with the previous findings of Portoghese and Olmsted.[72] For the reverse amidine series (**72-74**), an increase in the length of the alkyl chain appears to have little effect on the antagonist potency of the compounds at μ- and δ-receptors. Surprisingly, an increase in chain length appears to result in a slight decrease in antagonist potency at the κ-receptor. The propyl reverse amidine (**72**) is therefore the most κ-selective and potent compound in this series. In the previously discussed series of amidines (**133-135,70**) and amides (**130-132**), the ligands having the longest side chains showed increased selectivity for the κ-receptor as compared to their shorter chain homologues. It is entirely possible, that in this series of reverse amidines (**72-74**), the pentyl (**73**) and in particular the heptyl (**74**) analogues have exceeded the ideal side chain length.

K_i values are once again 5-10 fold higher than K_e values for the μ- and δ-receptors. For the κ-receptor, K_i values are approximately equal to K_e values for the n-alkyl amidine series (**133-135,70**). For amidines (**71-74**) however, K_i values are approximately 5 fold higher than K_e values. Shortly after the binding studies were completed on amidines (**133-135,70**), the κ-cell line died and had to be replaced. The K_i values for amidines (**71-74**) were determined with the new cell line. It is possible that if the binding assays were performed with the same cell line, the affinities for the κ-receptor would remain relatively constant as seen in the functional assay.

4.4.3 IMIDAZOLINE SUBSTITUENTS

Compounds (**83-84**) (**Table 33**) were prepared as described in section 2.3 and submitted for pharmacological evaluation as the hydrochloride salts. These were compared to compound (**82**), prepared by Jales[74] and submitted for pharmacological evaluation as the hydrochloride salt.



Compound	R^1	R^2	BU Number
82*	H	H	98027
83	CH_3	H	99019
84	CH_3	CH_3	99020

*from reference 74

Table 33 Imidazoline substituted compounds submitted for pharmacological testing

The binding affinities of the compounds were measured in cloned human opioid receptors (**Table 34**). Data provided by NIDA Opiate Treatment Drug Discovery Program (under contract to SRI)

	K_i (nM) ± SEM					
Compound	μ [^{3}H]-DAMGO	δ [^{3}H]CI-DPDPE	κ [^{3}H]U69,593	n	μ/κ	δ/κ
82	2.31 ± 0.18	4.72 ± 1.26	0.07 ± 0.01	2	33	67
83	19.41 ± 4.14	21.09 ± 0.33	1.39 ± 0.32	2	14	15
84	36.96 ± 12.06	45.18 ± 7.75	1.97 ± 0.20	2	19	23
40, norBNI	21.0 ± 5.0	5.7 ± 0.9	0.20 ± 0.05	2	105	28

from reference 74

Table 34 Binding affinities measured in cloned human opioid receptors

[^{35}S] GTPγS assays were performed in cloned human opioid receptors (**Table 35**). Data provided by NIDA Opiate Treatment Drug Discovery Program (under contract to SRI)

	K_e (nM) ± SEM					
Compound	μ-CHO membranes DAMGO	δ-CHO membranes DPDPE	κ-CHO membranes U69,593	n	μ/κ	δ/κ
82	2.85 ± 0.36	3.20 ± 0.78	0.22 ± 0.03	4	13	15
83	14.90 ± 2.08	8.87 ± 1.38	0.63 ± 0.03	5	24	14
84	24.17 ± 0.97	28.78 ± 3.05	1.14 ± 0.10	5	21	25
40, norBNI	18.9 ± 1.8	4.42 ± 0.38	0.04 ± 0.004		484	113

from reference 74

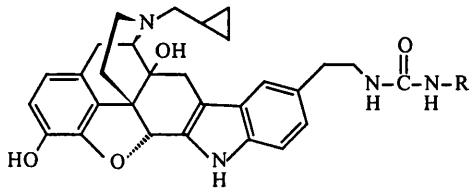
Table 35 [^{35}S] GTPγS assays performed in cloned human opioid receptors

The methyl (**83**) and dimethyl (**84**) analogues of (**82**) were synthesised in an attempt to further investigate the influence of lipophilicity on κ-selectivity and affinity. The general trend that can be established from binding assay results is that increased substitution of the imidazoline ring decreases affinity at μ-, δ- and κ-opioid receptors. Each of these compounds shows selectivity for the κ-receptor. In the binding assay, the unsubstituted imidazoline (**82**) appears to be the most selective compound. The methyl imidazoline (**83**) can be seen as a constrained analogue of the propyl reverse amidine (**72**). Comparison of the binding data for these two compounds show that while affinity for the μ- and κ-receptors is approximately the same, the affinity of the methyl imidazoline (**83**) for the δ-receptor is significantly lower than that of the equivalent propyl reverse amidine (**6**), resulting in greater κ- over δ-selectivity.

Results for the GTPγS assays were in keeping with the binding assay results. Thus, increased substitution of the imidazoline ring caused a decrease in antagonist potency at all three receptor types (**84** < **83** < **82**). The decrease was however less pronounced for the κ-receptor, which resulted in the methyl (**83**) and dimethyl (**84**) analogues showing slightly greater κ-selectivity. Again, this may indicate greater tolerance for steric bulk within the κ-receptor. The K_i values in this instance were not significantly different from the K_e values, except for compound (**82**) at the κ-receptor. It should be pointed out that this result was obtained with the previous cell line (*cf.* section 4.4.2). When comparing the GTPγS assay data for the constrained methyl imidazoline (**83**) and the non-constrained propyl reverse amidine analogue (**72**), it can be seen that the amidine (**72**) displays higher antagonist potency at all three receptor types, but particularly at the κ-receptor. In functional assays therefore, the propyl reverse amidine (**72**) shows greater selectivity and antagonist potency at the κ-opioid receptor than the methyl imidazoline (**83**).

4.4.4 UREA SUBSTITUENTS

The urea derivatives (**85–87**) (**Table 36**) were prepared as described in section 2.4 and submitted for pharmacological evaluation as the hydrochloride salts.



Compound	R	BU Number
85	CH_2CH_3	20003
86	$(CH_2)_3CH_3$	20002
87	$(CH_2)_5CH_3$	99038

Table 36 Urea substituted compounds submitted for pharmacological testing

The binding affinities of the compounds were measured in cloned human opioid receptors (**Table 37**). Data provided by NIDA Opiate Treatment Drug Discovery Program (under contract to SRI)

	K_i (nM) ± SEM					
Compound	μ [^{3}H]-DAMGO	δ [^{3}H]Cl-DPDPE	κ [^{3}H]U69,593	n	μ/κ	δ/κ
85	37.64 ± 14.41	2.43 ± 0.46	12.32 ± 1.29	2	3	0.2
86	4.80 ± 0.91	2.60 ± 0.54	6.33 ± 0.40	2	0.8	0.4
87	13.58 ± 4.06	2.25 ± 0.16	8.13 ± 2.67	2	2	0.3
40, norBNI	21.0 ± 5.0	5.7 ± 0.9	0.20 ± 0.05	2	105	28

Table 37 Binding affinities measured in cloned human opioid receptors

[^{35}S] GTPγS assays were performed in cloned human opioid receptors (**Table 38**). Data provided by NIDA Opiate Treatment Drug Discovery Program (under contract to SRI)

	K_e (nM) ± SEM					
Compound	μ-CHO membranes DAMGO	δ-CHO membranes DPDPE	κ-CHO membranes U69,593	n	μ/κ	δ/κ
85	1.60 ± 0.15	0.65 ± 0.02	2.47 ± 0.20	4	0.6	0.3
86	1.63 ± 0.12	0.53 ± 0.08	1.52 ± 0.16	4	1	0.3
87	1.79 ± 0.32	1.04 ± 0.18	1.71 ± 0.16	4	1	0.6
40, norBNI	18.9 ± 1.8	4.42 ± 0.38	0.04 ± 0.004		484	113

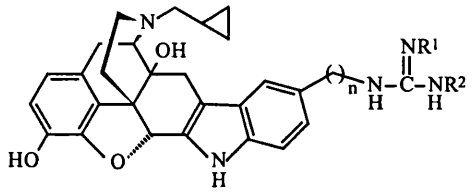
Table 38 [^{35}S] GTPγS assays performed in cloned human opioid receptors

The above urea derivatives were synthesised primarily in order to investigate the degree of basicity required for κ-selectivity. In binding assays, the three urea compounds (**85-87**) all had affinities of 2-3 nM for the δ-receptor, whereas the affinities for the κ-receptor ranged from 6-12 nM. The affinity of the butyl urea (**86**) for the κ-receptor was slightly greater than the affinity of either the ethyl (**85**) or hexyl (**87**) analogues. This effect was even more pronounced for the μ-receptor. The most significant finding is that although these compounds are essentially non-selective, a small degree of δ-selectivity was evident. This is surprising since the urea substituent is not obviously very different to an amide or amidine substituent. The observed change in selectivity has been attributed to an interaction between the carbonyl group and a basic Lys residue in the helix bundle of the δ-receptor (see section 3).

When comparing GTPγS assay results for the urea series (**85-87**), no significant change in the antagonist potency at μ-, δ- or κ-receptors can be seen. The three urea ligands appear equipotent and as found in the binding assays, slightly δ-selective. As shown for many of the ligands above, there is approximately a 5 fold difference between K_i and K_e values (although slightly greater deviation for μ values).

4.4.5 GUANIDINE SUBSTITUENTS

Compounds (**104–117**) (**Table 39**) were prepared as described in section 2.6 and submitted for pharmacological evaluation as the trifluoroacetic acid salts.



Compound	n	R^1	R^2	BU Number
104	2	H	H	20031
106	2	H	Benzyl	20022
110	2	H	*p*-Chlorobenzyl	20023
111	2	H	*p*-Nitrobenzyl	20024
112	2	H	*p*-Aminobenzyl	20025
105	0	H	Benzyl	20029
107	0	H	*p*-Chlorobenzyl	20030
108	0	H	*p*-Nitrobenzyl	01017
109	0	H	*p*-Aminobenzyl	01018
113	2	Butyl	Butyl	01010
114	0	Butyl	Butyl	01020
115	0	Propyl	Propyl	01021
116	0	Propyl	Cyclopropylmethyl	01022
117	0	Benzyl	Cyclopropylmethyl	01023

Table 39 Guanidine substituted compounds submitted for pharmacological testing

The binding affinities of the compounds were measured in cloned human opioid receptors (**Table 40**). Data provided by NIDA Opiate Treatment Drug Discovery Program (under contract to SRI)

	K_i (nM) ± SEM					
Compound	μ [^{3}H]-DAMGO	δ [^{3}H]Cl-DPDPE	κ [^{3}H]U69,593	n	μ/κ	δ/κ
104	5.69 ± 1.28	4.93 ± 1.28	0.49 ± 0.00	2	12	10
106	3.54 ± 0.25	7.24 ± 0.86	1.42 ± 0.17	2	2	5
110	7.74 ± 1.98	19.18 ± 0.17	2.41 ± 0.22	2	3	8
111	9.21 ± 3.73	11.70 ± 0.28	2.14 ± 0.34	2	4	5
112	7.78 ± 2.71	5.05 ± 0.23	0.95 ± 0.04	2	8	5
105	10.47 ± 1.87	26.81 ± 6.47	0.86 ± 0.20	2	12	31
107	29.78 ± 0.50	85.05 ± 5.06	0.66 ± 0.05	2	45	129
108	RO[a]	RO[a]	RO[a]			
109	RO[a]	RO[a]	RO[a]			
113	RO[a]	RO[a]	RO[a]			
114	RO[a]	RO[a]	RO[a]			
115	RO[a]	RO[a]	RO[a]			
116	RO[a]	RO[a]	RO[a]			
117	RO[a]	RO[a]	RO[a]			
40, norBNI	21.0 ± 5.0	5.7 ± 0.9	0.20 ± 0.05	2	105	28

[a]RO = Results Outstanding

Table 40 Binding affinities measured in cloned human opioid receptors

[^{35}S] GTPγS assays were performed in cloned human opioid receptors (**Table 41**). Data provided by NIDA Opiate Treatment Drug Discovery Program (under contract to SRI)

	K_e (nM) ± SEM					
Compound	**μ-CHO membranes DAMGO**	**δ-CHO membranes DPDPE**	**κ-CHO membranes U69,593**	**n**	**μ/κ**	**δ/κ**
104	1.25 ± 0.12	0.88 ± 0.15	0.40 ± 0.06	5	3	2
106	2.94 ± 0.31	1.36 ± 0.10	0.13 ± 0.01	5	23	10
110	2.61 ± 0.41	1.48 ± 0.14	0.23 ± 0.02	5	11	6
111	2.20 ± 0.60	1.34 ± 0.24	0.17 ± 0.01	5	13	8
112	1.57 ± 0.13	0.95 ± 0.14	0.25 ± 0.03	5	6	4
105	1.41 ± 0.17	4.09 ± 0.63	0.06 ± 0.01	5	24	68
107	5.24 ± 1.13	7.67 ± 1.36	0.14 ± 0.01	5	37	55
108	RO[a]	RO[a]	RO[a]			
109	RO[a]	RO[a]	RO[a]			
113	RO[a]	RO[a]	RO[a]			
114	RO[a]	RO[a]	RO[a]			
115	RO[a]	RO[a]	RO[a]			
116	RO[a]	RO[a]	RO[a]			
117	RO[a]	RO[a]	RO[a]			
40, norBNI	18.9 ± 1.8	4.42 ± 0.38	0.04 ± 0.004		484	113

[a]RO = Results Outstanding

Table 41 [^{35}S] GTPγS assays performed in cloned human opioid receptors

From the guanidine series of compounds, we hoped to gain greater insight into the role of lipophilic interactions (hence the aromatic groups) as well as the optimum distance between the two basic nitrogen centres. The guanidine series (**106,110-112**) are benzyl substituted analogues of guanidine (**104**). While benzyl substitution appears to have had little effect on the affinity of the compounds for the μ-receptor, δ-opioid affinity is slightly reduced. Affinity for the κ-receptor also seems to have decreased by 3-5 fold. Within this series (**106,110-112**), the introduction of various *p*-substituents shows no effect on affinity. In the guanidine series (**105,107-109**), the two methylene groups between the guanidine functionality and the naltrindole core (*cf.* ligands **106,110-112**) have been removed, effectively bringing the two basic nitrogen groups closer together (*ie.* benzyl analogues of GNTI, **47**). Comparison of the data for benzylguanidines (**106,110-112**) and benzylguanidines (**105,107-109**) shows that although decreasing the distance between the naltrindole "message" and the basic benzylguanidinyl group has little effect on κ-affinity, affinity for both μ- and δ-receptors is decreased, in turn increasing κ-selectivity. This might suggest that an increase in steric bulk near to the

indolomorphinan moiety is better tolerated by the κ-receptor than by the μ- or δ-receptors, or that a basic functionality near to the indolomorphinan nucleus is detrimental to μ- and δ-affinity. This effect is greater for the *p*-chlorobenzyl substituent than for the benzyl substituent, perhaps suggesting that the effect is due to bulk/lipophilicity. It is hoped that the outstanding assay results would further confirm these hypotheses.

In GTPγS assays, compounds (**104-107,110-112**) displayed essentially equal and potent antagonist activity at the κ-receptor. Comparison of compounds (**104,106,110-112**) shows that the addition of a benzyl substituent to the guanidine group causes very slight decreases in μ- and δ- antagonist potency, resulting in overall increased κ-selectivity. When compared to the longer chain benzylguanidine (**106**), the shorter chain benzylguanidine analogue (**105**) shows slightly increased antagonist potency at the μ- and κ-receptors, with slightly decreased antagonist potency at the δ-receptor. Addition of a *p*-chloro substituent to the benzyl group (*cf.* **105** and **107**), causes a decrease in antagonist affinity for both the μ- and δ-receptors, resulting in increased κ- over μ- and κ- over δ-selectivity.

4.4.6 COMPARISON OF TESTING METHODS

Since the values obtained in binding and functional assays depend on the exact conditions used and may be subject to change (as discussed in section 4.4.2), we decided to compare the two compounds prepared by both ourselves and the Portoghese group.

Radio Ligand Binding

	K_i (nM) ± SEM				
Compound	μ	δ	κ	μ/κ	δ/κ
104	5.69 ± 1.28[a,g]	4.93 ± 1.28[b,g]	0.49 ± 0.00[c,g]	12	10
104	33.40 ± 4.70[d,h]	10.70 ± 3.00[e,h]	0.51 ± 0.13[f,h]	66	21
98	3.59 ± 1.30[a,g]	8.78 ± 0.37[b,g]	0.40 ± 0.14[c,g]	9	22
98	Not Reported	Not Reported	Not Reported		

[a][^{3}H]-DAMGO, [b][^{3}H]CI-DPDPE, [c][^{3}H]U69,593, [d]μ-HEK-293 [^{3}H]diprenorphine, [e]δ-HEK-293 [^{3}H]diprenorphine, [f]κ-HEK-293 [^{3}H]diprenorphine, [g]Data provided by NIDA OTDP (under contract to SRI), [h]Data from Stevens, *et al.*[77]

Table 42 Binding affinities

When comparing the results of the SRI binding assay to that of the Portoghese group, we can see that for guanidine (**104**), the affinity at the μ-receptor is 6 fold greater, the affinity at the δ-receptor is 2 fold greater and the affinity at the κ-receptor is unchanged. Compound (**104**) therefore shows lower κ- over μ-and κ- over δ-selectivity under SRI assay conditions. A comparison could not be made for (**98**), since no binding affinities were reported.

Functional Assays

	K_e (nM) ± SEM				
Compound	μ	δ	κ	μ/κ	δ/κ
104	1.25 ± 0.12[a,g]	0.88 ± 0.15[b,g]	0.40 ± 0.06[c,g]	3	2
104	12[d,h]	3.4[e,h]	1.7[f,h]	7	2
98	3.35 ± 0.80[a,g]	5.10 ± 1.01[b,g]	0.31 ± 0.05[c,g]	11	16
98	ND	ND	3.2		

[a]μ-CHO membranes-DAMGO, [b]δ-CHO membranes-DPDPE, [c]κ-CHO membranes-U69593, [d]μ-GPI-morphine, [e]δ-MVD-[D-Ala^2,D-Leu^5]enkephalin, [f]κ-GPI -ethylketazocine, [g]Data provided by NIDA OTDP (under contract to SRI), [h]Data from Stevens, *et al.*[77]

Table 43 Functional assays

In the SRI functional assays, guanidine (**104**) showed 4 fold greater antagonist potency at both the δ- and κ-receptors, than in the assays performed by the Portoghese group. This resulted in a similar κ- over δ-selectivity ratio for both assay conditions. Compound (**104**) however, showed 10 fold lower antagonist potency at the μ-receptor under Portoghese's assay conditions, which resulted in greater κ- over μ-selectivity being reported. A comparison between the two assay conditions could not be made for (**98**), since only data at the κ-receptor was reported. (**98**) is reported as being 10 fold less potent than determined under SRI assay conditions.

4.4.7 CONCLUSION

Little variation in κ-affinity was observed within, or even between the series of compounds synthesised. κ-Selectivity differences between series were largely a result of changes in affinity at δ and μ. This is in agreement with findings from the Portoghese group,[77] and can be explained by the negative interactions of the basic side chain with the basic Lys 303 residue (μ), or the bulky Trp 284 residue (δ), as discussed in section 3.2.3. These interactions were not specifically examined in the current project, but cursory examination of the models found the above mentioned residues to lie within regions which would make interaction with the side chain possible.

Results from the functional assays appear to be more consistent than those obtained in binding assays. This could be related to the fact that the number of experiments performed, (n), is greater for the antagonist functional assays. Deterioration of the radioligand used in binding assays could also conceivably influence binding assay results. In lieu of this, it may prove preferable to use the results of functional assays in future modelling studies, rather than the results of binding assays.

5. EXPERIMENTAL

5.1 ANALYTICAL SPECIFICATIONS

Column chromatography was performed under gravity, over silica gel 60 (35-70μm) purchased from Merck. Preparative TLC was performed on plates made with Kieselgel 60 $PF_{254+366}$ for prep. TLC, obtained from Merck. The thickness of the silica layer was approximately 1 mm. Analytical TLC was performed using aluminium-backed plates coated with Kieselgel 60 F_{254}, from Merck. The chromatograms were visualised using either UV light (UVGL-58, short wavelength), ninhydrin (acidic) or potassium permanganate (basic). Melting points were carried out using a Reichert-Jung Thermo Galen Kopfler block or a Gallenkamp MFB-595 melting point apparatus and are uncorrected. High and low resolution fast atom bombardment (FAB) mass spectra were recorded on a Fisons VG AutoSpec Q instrument, with a matrix of *m*-nitrobenzyl alcohol. High and low resolution electron impact (EI) mass spectra were recorded using EI ionisation at 70eV, on a VG AutoSpec instrument, equipped with a Fisons autosampler. ^{1}H NMR and ^{13}C NMR spectra were recorded using either JEOL 270 (operating at 270 MHz for ^{1}H and 67.8 MHz for ^{13}C); JEOL Lambda 300 (operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C) or JEOL EX 400 (operating at 400 MHz for ^{1}H and 100.5 MHz for ^{13}C) spectrometers. Chemical shifts (δ) are measured in ppm. Spectra were referenced internally using the residual solvent resonance. Coupling constants (J) are expressed in Hz and the multiplicities are abbreviated as follows: s (singlet), d (doublet), t (triplet), m (multiplet) and br (broad). Only diagnostic peaks have been quoted for proton NMR. Microanalysis was performed by the Microanalysis Laboratory in the Department of Chemistry, University of Bath. Analytical RP-HPLC was performed with a Beckman System Gold 125 solvent module, equipped with a Beckman System Gold 166 detector (λ = 254nm). The column stationary phase was Beckman ultrasphere ODS, 5 μm (15 cm x 4.6 mm). A mobile phase of [MeOH/0.3% NH_4CO_3 (80:20)] was used at a flow rate of 1 ml/min. Infrared spectroscopy was performed on either a Perkin-Elmer 782 Instrument, or on a Perkin-Elmer RX 1 FT-IR Instrument. Anhydrous THF, DMF, DCM and MeOH were purchased from Aldrich. HPLC solvent grade chloroform and MeOH were purchased from Merck. All other solvents used were GPR grade, purchased from Merck

or Fisher Scientific. Chemicals were purchased from Aldrich, Fluka, Lancaster and Acros chemical companies.

5.2 GENERAL PROCEDURES

General Procedure A – Aromatic Amides

5'-Carboxy-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-dihydroxyindolo[2',3':6,7]-morphinan (**64**) (1 eq) in anhydrous CH_2Cl_2 at 0 °C was brought into solution by the dropwise addition of triethylamine. To this solution was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (1.5 eq) and 4-dimethylaminopyridine (DMAP) (catalytic). The resultant mixture was allowed to stir for 20 min, after which the aromatic amine (1.5 eq) was added. After stirring at RT for 12 h, CH_2Cl_2 (10 ml) was added and the mixture further basified with excess aq.$NaHCO_3$. The mixture was then washed with brine, dried (Na_2SO_4), filtered and concentrated, to give the crude product which was further purified by preparative thin layer chromatography [$CH_3OH/CH_2Cl_2/NH_4OH$ (20:80:1)] with typical yields of 9-22%.

General Procedure B – Reverse Amidines

The appropriate amine (1.3 eq) was added portionwise to the thioimidic ester derivative (1 eq) (**78**) in anhydrous CH_3OH at RT. After stirring for 12 h, the solution was concentrated *in vacuo* and the residue treated with Et_2O. The amidinium salt was filtered and washed with Et_2O. The products were purified by preparative thin layer chromatography [$CH_3OH/CH_2Cl_2/NH_4OH$ (20:80:1)]. The major product was the required reverse amidine. A minor product was the disubstituted reverse amidine.

General Procedure C - Imidazolines

p-Toluenesulfonic acid (*p*-TSA) (1 eq) was stirred in ^{i}PrOH (50 ml) at RT. The required diamine was added, after which the solution was stirred for 30 min before being evaporated,

recrystallised from hot ^{i}PrOH, filtered and washed with CH_2Cl_2 and petroleum ether.[74] The *p*-TSA salt of the diamine was added to 5'-cyanomethyl-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-dihydroxyindolo[2',3':6,7]morphinan (**75**) under N_2. The mixture was heated to 160 °C for 3 h then allowed to cool, after which H_2O (2 ml) was added and the mixture basified with 2N NaOH (pH 10). After extraction with $CHCl_3$, the organic extracts were washed, dried (Na_2SO_4), filtered and concentrated. Further purification by preparative thin layer chromatography [$CH_3OH/CH_2Cl_2/NH_4OH$ (20:75:1)] afforded the required imidazoline in yields of 5-6%.

General Procedure D - Ureas

5'-Aminoethyl-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-dihydroxyindolo[2',3':6,7]-morphinan (**76**) (1 eq) in anhydrous [CH_2Cl_2/CH_3OH (10:1)] (20 ml) was added dropwise to an ice-bath cooled solution of the appropriate isocyanate (1 eq) in anhydrous CH_2Cl_2 (20 ml). After the addition was complete, the solution was allowed to stir at RT for 5 h, after which the solvent was removed *in vacuo* and the crude residue purified by preparative thin layer chromatography [$CH_3OH/CH_2Cl_2/NH_4OH$ (20:80:1)], giving the desired product in a yield of 30-40%. A minor product in which the phenolic OH additionally reacted with the isocyanate to form the carbamate was also isolated.

General Procedure E - N'-Benzylguanidinylating agents

1,3-Bis-Boc-2-methyl-2-thiopseudourea (1 eq) was dissolved in anhydrous *N,N*-dimethylformamide (20 ml) and cooled in an ice bath. Sodium hydride (1.2 eq) was added in a single portion and the solution stirred at this temperature for 1 h. The appropriate benzyl bromide (1.1 eq) was added in a single portion and the solution allowed to stir for 12 h at RT. The reaction was quenched with water (30 ml) and extracted three times with ethyl acetate. The combined organic layers were washed twice with brine, dried (Na_2SO_4), filtered and concentrated to give the crude guanydinylating agent. This crude mixture was subjected to column chromatography [hexane/EtOAc (9:2)] providing the desired guanidinylating agent in yields of 65 - 80%.

General Procedure F - Guanidinylation

The required amine (1 eq), guanylating agent (1.3-2.0 eq), triethylamine (1-2 eq) and mercury(II)chloride (1.0-1.5 eq) were all added to anhydrous *N,N*-dimethylformamide (20ml) in an ice bath under a nitrogen atmosphere. The solution was then stirred at 50 °C for 12-24 h. The solution was subsequently filtered to remove any solid and the reaction quenched by the addition of sodium bicarbonate (30 ml). The solution was extracted with ethyl acetate and then washed successively with water and brine, dried (Na_2SO_4), filtered and concentrated under reduced pressure to give the crude product mixture, which was purified by column chromatography - gradient elution (CH_2Cl_2) until unreacted guanidinylating agent has been removed then [$CH_3OH/CH_2Cl_2/NH_4OH$ (10:89:1)], affording the desired products in 25-68% yields.

General Procedure G - Removing BOC

The appropriate BOC-protected benzyl guanidinylated amine was dissolved in dichloromethane (4ml) and allowed to stir for 10 min at 0 °C. Trifluoroacetic acid (2ml) was added and the solution allowed to warm to room temperature. Stirring was continued for 12 h, after which the solution was concentrated under reduced pressure. Washing the resultant oil with diethyl ether afforded a precipitate that could be isolated by vacuum filtration. Further purification was achieved by recrystallization (methanol/diethyl ether).

General Procedure H – N,N'-disubstituted thioureas

Calcium carbonate (1eq) was dissolved in H_2O (2ml) and added to a stirred solution of the required amine (1 eq) in $CHCl_3$ (30 ml). Thiophosgene (2 eq) was added and the solution stirred at RT for 24 h. The aqueous layer was washed with H_2O, and concentrated to give the required isothiocyanate (purity was checked by TLC and no further purification was needed). The isothiocyanate (1eq) was then dissolved in acetone (15 ml) and added dropwise to the required amine (1 eq) in acetone (15 ml). The solution was refluxed gently for 3 h, concentrated

and purified by column chromatography [hexane/EtOAc (1:1)] providing the desired N,N'-disubstituted thiourea in yields of 46 - 56%.

General Procedure I – BOC-protection of N,N'-disubstituted thioureas

To NaH (2 eq) (prewashed with THF) in THF (40 ml) at 0 °C, was added the appropriate N,N'-disubstituted thiourea (1eq). The mixture was stirred for 10 min, after which di-*tert*-butyl-dicarbonate (BOC-anhydride) (1.15 eq) in THF (10 ml) was added. The mixture was then allowed to warm to RT and stirred for a further 12 h. The reaction mixture was quenched by stirring with 10% NaOH for 20 min. The organic layer was then separated and the aqueous layer further extracted with EtOAc. The combined organic layers were concentrated and subsequently treated with hexane, which caused unreacted starting material to precipitate out. The crystals were removed by filtration and the filtrate subsequently purified by column chromotography [EtOAc/hexane (1:3)], yielding the mono- or di-N,N'-disubstituted thiourea in yields of 45-60%.

5.3 SYNTHETIC METHODS

5'-Carboxy-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-dihydroxyindolo-[2',3':6,7]morphinan (64)[72]

Naltrexone hydrochloride (2.34 g, 6.2 mmol) and 4-hydrazinobenzoic acid (1.09 g, 7.2 mmol) were dissolved in glacial acetic acid (100 ml) and stirred under nitrogen at 85 °C for 72 h. The mixture was then cooled and filtered, and the crude product washed with glacial acetic acid, acetone and then ether. The solid was dissolved in methanol, dried (Na_2SO_4), filtered and concentrated *in vacuo* to give 5'-carboxy-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-dihydroxyindolo[2',3':6,7]morphinan (**64**) (as the hydrochloride salt) 1.90 g, 3.8 mmol, 62%; IR ν_{max}/cm (KBr) 1680 (C=O) (Lit.[77] 1679); ^{1}H NMR (400 MHz, CD_3OD) δ 0.38-0.49 [m, 2H, $NCH_2CH(CHHCHH)$], 0.63-0.82 [m, 2H, $NCH_2CH(CHHCHH)$], 0.97-1.04 [m, 1H, $NCH_2CH(CH_2CH_2)$], 5.65 [s, 1H, C(5)*H*], 6.79 [d, J=8.4 Hz, 1H, C(1)*H*], 6.82 [d, J=8.4 Hz, 1H,

C(2)*H*], 7.33 [d, J=8.4 Hz, 1H, C(7')*H*], 7.68 [d, J=8.4 Hz, 1H, C(6')*H*] and 8.09 [s, 1H, *C*(4')]; ^{13}C NMR (67.8 MHz, CD_3OD) δ 3.6 [$NCH_2CH(CH_2CH_2)$], 6.6 [$NCH_2CH(CH_2CH_2)$], 7.0 [$NCH_2CH(CH_2CH_2)$], 25.2 [*C*(10)], 29.9 [*C*(15)], 30.2 [*C*(8)], 47.5 [*C*(16)], 48.1 [quaternary *C*(13)], 59.0 [*C*(18)], 63.6 [*C*(9)], 73.9 [quaternary *C*(14)], 84.9 [*C*(5)], 111.2 [*C*(7')], 112.6 (quaternary Ar), 119.8 [*C*(2)], 121.5 [*C*(4')], 122.5 [*C*(1)], 123.0 [*C*(6')], 123.2 (quaternary Ar), 125.3 (quaternary Ar), 127.6 (quaternary Ar), 130.4 (quaternary Ar), 132.5 (quaternary Ar), 141.6 (quaternary Ar), 142.2 (quaternary Ar), 144.9 (quaternary Ar) and 171.4 (*C*=O); FAB-MS *m/z* 459 [$(m+1)^+$, 100%], 441 (10).

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N-heptyl)amido-3,14-dihydroxyindolo-[2',3':6,7]morphinan (59)

5'-Carboxy-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-dihydroxy-indolo[2',3':6,7]-morphinan (**64**) (0.033 g, 0.067 mmol) in anhydrous CH_2Cl_2 (5 ml) was brought into solution by the dropwise addition of triethylamine. (BOP) reagent (30 mg, 0.068 mmol) and heptylamine (0.010 g, 0.013 ml, 0.088 mmol) were added, after which the solution was stirred at 25 °C for 24 h. The reaction mixture was then added to ethyl acetate (30 ml) and washed 3 times with brine (pH 10). The organic phase was dried (Na_2SO_4), filtered and concentrated to yield 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N-heptyl)amido-3,14-dihydroxyindolo[2',3':6,7]-morphinan (**59**) (31 mg, 0.056 mmol, 83%), which was then converted to the hydrochloride salt, mp. 181-183 °C; R_f = 0.66 [$CH_3OH/CH_2Cl_2/NH_4OH$ (20:80:1)]; HPLC (C_{18} column) 5.296 [MeOH/0.3% NH_4CO_3 (80:20)]; IR ν_{max}/cm (KBr) 3640-2460 (br, bonded OH and amide NH), 1620 (amide I) and 1540 (amide II); ^{1}H NMR (270 MHz, CD_3OD) δ 0.06-0.10 [m, 2H, $NCH_2CH(CHHCHH)$], 0.41-0.50 [m, 2H, $NCH_2CH(CHHCHH)$], 0.75-0.79 (m, 3H, CH_3), 0.79-0.84 [m, 1H, $NCH_2CH(CH_2CH_2)$], 5.46 [s, 1H, C(5)*H*], 6.41-6.49 [m, 2H, C(1)*H* and C(2)*H*], 7.21 [d, J=8.7 Hz, 1H, C(7')*H*], 7.48 [dd, J_1=8.7 Hz, J_2=1.6 Hz, 1H, C(6')*H*] and 7.83 [d, J=1.6 Hz, 1H, C(4')*H*]; ^{13}C NMR (67.8 MHz, CD_3OD) δ 2.8 [$NCH_2CH(CH_2CH_2)$], 3.3 [$NCH_2CH(CH_2CH_2)$], 8.9 [$NCH_2CH(CH_2CH_2)$], 13.0 (CH_3), 22.3 [*C*(10)], 22.8, 26.4, 26.8, 28.8, 31.6 ($NHCH_2CH_2CH_2CH_2CH_2CH_2CH_3$), 29.3 [*C*(15)], 31.4 [*C*(8)], 40.2 ($CONHCH_2$), 43.7 [*C*(16)], 47.6 [quaternary *C*(13)], 59.1 [*C*(18)], 62.3 [*C*(9)], 73.1 [quaternary *C*(14)], 84.4 [*C*(5)], 110.7 [*C*(7')],

111.2 (quaternary Ar), 117.2 [*C*(2)], 118.3 [*C*(4')], 118.6 [*C*(1)], 121.2 [*C*(6')], 124.8 (quaternary Ar), 125.0 (quaternary Ar), 126.3 (quaternary Ar), 130.7 (quaternary Ar), 131.3 (quaternary Ar), 139.2 (quaternary Ar), 139.8 (quaternary Ar), 143.4 (quaternary Ar) and 170.2 (*C*=O); EI-MS *m/z* 555 [$(M)^+$, 85%], 500 (15) and 441 (15); HRMS (EI) *m/z* 555.3099 $(M)^+$, $C_{34}H_{41}N_3O_4$ requires 555.3097.

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N-benzyl)amido-3,14-dihydroxy-indolo[2',3':6,7]morphinan (60)

5'-Carboxy-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-dihydroxy-indolo[2',3':6,7]-morphinan (**64**) (0.201 g, 0.407 mmol) and benzylamine (0.064 g, 0.066 ml, 0.598 mmol) were reacted according to general procedure A, to yield 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N-benzyl)amido-3,14-dihydroxyindolo[2',3':6,7]morphinan (**60**) (0.045 g, 0.083 mmol, 21%). The product was then converted to the hydrochloride salt, mp. 166-169 °C; R_f = 0.45 [$CH_3OH/CH_2Cl_2/NH_4OH$ (10:89:1)]; HPLC (C_{18} column) 1.767 [MeOH/0.3% NH_4CO_3 (80:20)]; IR ν_{max}/cm (KBr) 3620-2480 (br, bonded OH and amide NH), 1640 (amide I) and 1560 (amide II); ^1H NMR (270 MHz, CD_3OD) δ 0.49-0.60 [m, 2H, $NCH_2CH(CHHCHH)$], 0.88 [m, 2H, $NCH_2CH(CHHCHH)$], 1.28 [m, 1H, $NCH_2CH(CH_2CH_2)$], 5.71 [s, 1H, C(5)*H*], 6.66-6.68 [m, 2H, C(1)*H* and C(2)*H*], 7.18-7.37 [m, 5H, ArH], 7.40 [d, J=8.7 Hz, 1H, C(7')*H*], 7.65 [dd, J_1=8.7 Hz, J_2=1.3 Hz, 1H, C(6')*H*] and 8.01 [d, J=1.3 Hz, 1H, C(4')*H*]; ^{13}C NMR (67.8 MHz, CD_3OD) δ 2.1 [$NCH_2CH(CH_2CH_2)$], 4.9 [$NCH_2CH(CH_2CH_2)$], 5.6 [$NCH_2CH(CH_2CH_2)$], 23.7 [*C*(10)], 28.5 [*C*(15)], 28.9 [*C*(8)], 43.3 ($CONHCH_2$), 46.3 [*C*(16)], 46.8 [quaternary *C*(13)], 57.7 [*C*(18)], 62.6 [*C*(9)], 72.4 [quaternary *C*(14)], 83.6 [*C*(5)], 109.5 (Ar), 111.0 (Ar), 118.3 (Ar), 118.7 (Ar), 119.3 (Ar), 121.3 (Ar), 121.5 (Ar), 125.1 (Ar), 126.3 (Ar), 126.7 (Ar), 127.2 (2 x Ar), 128.2 (2 x Ar), 128.9 (Ar), 130.8 (Ar), 139.2 (Ar), 139.5 (Ar), 140.8 (Ar), 143.4 (Ar) and 170.0 (*C*=O); EI-MS *m/z* 547 [$(M)^+$, 100%], 506 (30) and 441 (10); HRMS (EI) *m/z* 547.2476 $(M)^+$, $C_{34}H_{33}N_3O_4$ requires 547.2471.

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N-phenethyl)amido-3,14-dihydroxy-indolo[2',3':6,7]morphinan (61)

5'-Carboxy-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-dihydroxy-indolo[2',3':6,7]-morphinan (**64**) (0.204 g, 0.413 mmol) and phenethylamine (0.073 g, 0.076 ml, 0.607 mmol) were reacted according to general procedure A, to yield 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N-phenethyl)amido-3,14-dihydroxyindolo[2',3':6,7]morphinan (**61**) (0.021 g, 0.038 mmol, 9%). The product was then converted to the hydrochloride salt, mp. 178 °C; R_f = 0.55 [$CH_3OH/CH_2Cl_2/NH_4OH$ (10:89:1)]; HPLC (C_{18} column) 1.850 [MeOH/0.3% NH_4CO_3 (80:20)]; IR ν_{max}/cm (KBr) 3660-2820 (br, bonded OH and amide NH), 1640 (amide I) and 1550 (amide II); ^{1}H NMR (270 MHz, CD_3OD) δ 0.24-0.27 [m, 2H, $NCH_2CH(CHHCHH)$], 0.54-0.69 [m, 2H, $NCH_2CH(CHHCHH)$], 0.88-0.94 [m, 1H, $NCH_2CH(CH_2CH_2)$], 5.60 [s, 1H, C(5)*H*], 6.54-6.60 [m, 2H, C(1)*H* and C(2)*H*], 7.13 – 7.30 (m, 5H, Ar*H*), 7.34 [d, J=8.7 Hz, 1H, C(7')*H*], 7.56 [dd, J_1=8.7 Hz, J_2=1.6 Hz, 1H, C(6')*H*] and 7.91 [d, J=1.6 Hz, 1H, C(4')*H*]; ^{13}C NMR (67.8 MHz, CD_3OD) δ 2.7 [$NCH_2CH(CH_2CH_2)$], 3.5 [$NCH_2CH(CH_2CH_2)$], 8.4 [$NCH_2CH(CH_2CH_2)$], 23.0 [*C*(10)], 28.5 [*C*(15)], 31.1 [*C*(8)], 35.4 ($CONHCH_2CH_2$), 41.4 ($CONHCH_2$), 44.2 [*C*(16)], 47.5 [quaternary *C*(13)], 58.9 [*C*(18)], 62.4 [*C*(9)], 73.0 [quaternary *C*(14)], 84.3 [*C*(5)], 110.7 [*C*(7')], 110.9 (quaternary Ar), 117.3 [*C*(2)], 118.4 [*C*(4')], 118.7 [*C*(1)], 121.2 [*C*(6')], 124.4 (Ar), 125.0 (quaternary Ar), 126.0 (quaternary Ar), 126.3 (quaternary Ar), 128.1 (2 x Ar), 128.5 (2 x Ar), 130.6 (quaternary Ar), 131.2 (quaternary Ar), 139.3 (quaternary Ar), 139.4 (quaternary Ar), 139.9 (Ar), 143.4 (quaternary Ar) and 170.2 (*C*=O); EI-MS *m/z* 561 [$(M)^+$, 100%], 520 (20) and 441 (20); HRMS (EI) *m/z* 561.2626 $(M)^+$, $C_{35}H_{35}N_3O_4$ requires 561.2628.

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N-phenylbutyl)amido-3,14-dihydroxy-indolo[2',3':6,7]morphinan (62)

5'-Carboxy-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-dihydroxy-indolo[2',3':6,7]-morphinan (**64**) (0.100 g, 0.202 mmol) and phenylbutylamine (0.045 g, 0.048 ml, 0.302 mmol) were reacted according to general procedure A, to yield 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N-phenylbutyl)amido-3,14-dihydroxyindolo[2',3':6,7]morphinan (**62**) (0.027 g,

0.045 mmol, 15%). The product was then converted to the hydrochloride salt, mp. >220 °C; R_f = 0.51 [$CH_3OH/CH_2Cl_2/NH_4OH$ (10:89:1)]; HPLC (C_{18} column) 1.672 [MeOH/0.3% NH_4CO_3 (80:20)]; IR ν_{max}/cm (KBr) 3640-2840 (br, bonded OH and amide NH), 1620 (amide I) and 1530 (amide II); ^{1}H NMR (300 MHz, CD_3OD) δ 0.49-0.58 [m, 2H, $NCH_2CH(CHHCHH)$], 0.73-0.80 [m, 2H, $NCH_2CH(CHHCHH)$], 0.83-0.92 [m, 1H, $NCH_2CH(CH_2CH_2)$], 5.70 [s, 1H, C(5)*H*], 6.65-6.71 [m, 2H, C(1)*H* and C(2)*H*], 7.07 – 7.24 (m, 5H, Ar*H*), 7.39 [d, J=8.6 Hz, 1H, C(7')*H*], 7.59 [dd, J_1=8.6 Hz, J_2=1.6 Hz, 1H, C(6')*H*] and 7.95 [d, J=1.6 Hz, 1H, C(4')*H*]; ^{13}C NMR (75 MHz, CD_3OD) δ 3.4 [$NCH_2CH(CH_2CH_2)$], 6.3 [$NCH_2CH(CH_2CH_2)$], 6.8 [$NCH_2CH(CH_2CH_2)$], 25.0 [*C*(10)], 29.8 [*C*(15)], 30.1 [*C*(8)], 30.2, 36.5 and 36.5 ($NHCH_2CH_2CH_2CH_2$), 41.1 ($CONHCH_2$), 47.6 [*C*(16)], 48.7 [quaternary *C*(13)], 58.9 [*C*(18)], 63.7 [*C*(9)], 73.7 [quaternary *C*(14)], 84.8 [*C*(5)], 110.8 (quaternary Ar) , 112.3 [*C*(7')], 119.5 [*C*(2)], 120.0 [*C*(4')], 120.7 [*C*(1)], 122.6 (quaternary Ar), 122.7 [*C*(6')], 126.0 (quaternary Ar), 126.7 (Ar), 127.6 (quaternary Ar), 129.3 (2 x Ar), 129.4 (2 x Ar), 130.2 (quaternary Ar), 132.2 (quaternary Ar), 140.8 (quaternary Ar), 142.1 (quaternary Ar), 143.6 (quaternary Ar), 144.9 (quaternary Ar) and 171.7 (*C*=O); EI-MS *m/z* 589 [$(M)^+$, 100%], 548 (20) and 441 (10); HRMS (EI) *m/z* 589.2932 $(M)^+$, $C_{37}H_{39}N_3O_4$ requires 589.2941.

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N-4-methoxybenzyl)amido-3,14-dihydroxyindolo[2',3':6,7]morphinan (63)

5'-Carboxy-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-dihydroxy-indolo[2',3':6,7]-morphinan (**64**) (0.101 g, 0.204 mmol) and 4-methoxybenzylamine (0.042 g, 0.040 ml, 0.306 mmol) were reacted according to general procedure A, to yield 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N-4"-methoxybenzyl)amido-3,14-dihydroxyindolo[2',3':6,7]morphinan (**63**) (0.031 g, 0.054 mmol, 26%). The product was then converted to the hydrochloride salt, mp. >220 °C (dec); R_f = 0.68 [$CH_3OH/CH_2Cl_2/NH_4OH$ (20:80:1)]; HPLC (C_{18} column) 2.633 [MeOH/0.3% NH_4CO_3 (80:20)]; IR ν_{max}/cm (KBr) 3680-2580 (br, bonded OH and amide NH), 1660 (amide I) and 1520 (amide II); ^{1}H NMR (270 MHz, CD_3OD) δ 0.29-0.45 [m, 2H, $NCH_2CH(CHHCHH)$], 0.53-0.77 [m, 2H, $NCH_2CH(CHHCHH)$], 0.86-0.98 [m, 1H, $NCH_2CH(CH_2CH_2)$], 5.53 [s, 1H, C(5)*H*], 6.51-6.63 [m, 2H, C(1)*H* and C(2)*H*], 6.68 (d, J=8.8 Hz,

2H, C(3")*H* and C(5")*H*), 7.12 (d, J=8.8 Hz, 2H, C(2")*H* and C(6")*H*), 7.23 [d, J=9.2 Hz, 1H, C(7')*H*], 7.48 [d, J=9.2 Hz, 1H, C(6')*H*] and 7.82 [s, 1H, C(4')*H*]; ^{13}C NMR (67.8 MHz, CD_3OD) δ 3.8 [$NCH_2CH(CH_2CH_2)$], 6.6 [$NCH_2CH(CH_2CH_2)$], 7.1 [$NCH_2CH(CH_2CH_2)$], 25.3 [*C*(10)], 30.1 [*C*(15)], 30.5 [*C*(8)], 44.4 ($CONHCH_2$), 47.7 [*C*(16)], 48.4 [quaternary *C*(13)], 50.3 (OCH_3), 56.0 [*C*(18)], 63.9 [*C*(9)], 74.0 [quaternary *C*(14)], 85.1 [*C*(5)], 111.1 (quaternary Ar), 112.7 [*C*(7')], 115.2 [*C*(3") and *C*(5")], 119.8 [*C*(2)], 120.4 (Ar), 121.2 (Ar), 123.0 (quaternary Ar), 123.1 [*C*(6')], 126.5 (quaternary Ar), 127.8 (quaternary Ar), 130.2 [*C*(2") and *C*(6")], 130.5 (quaternary Ar), 132.5 (quaternary Ar), 132.7 (quaternary Ar), 141.0 (quaternary Ar), 142.3 (quaternary Ar), 145.1 (quaternary Ar), 160.6 [C(4")] and 171.5 (*C*=O); EI-MS *m/z* 577 [$(M)^+$, 100%], 536 (20) and 441 (10).

4-Hydrazinobenzyl cyanide[107]

A solution of 4-aminobenzylcyanide (1.20 g, 9.09 mmol) in c.HCl (22.5 ml) and H_2O (32 ml) was cooled in an ice-bath. Sodium nitrite (0.71 g, 10.29 mmol) in H_2O (10 ml) was added dropwise with stirring. After stirring at 0 °C for 30 min, the diazonium salt was added to $SnCl_2.H_2O$ (7.91 g) in H_2O (75 ml) at 0 °C and left to stand at 0 °C for 1 h. The precipitate was filtered off and washed successively with ether, hexane and petroleum ether. After basification with NH_4OH, extraction with dichloromethane gave 4-hydrazinobenzyl cyanide (0.89 g, 6.02 mmol, 66%) mp. 73.1-75.2 °C (Lit.[107] 68-70 °C) ; IR ν_{max}/cm ($CHCl_3$) 3370-3003 (br, NH_2NH) and 2253 (CN); ^{1}H NMR (270 MHz, $CDCl_3$) δ 3.59 (br s, 1H, N*H*), 3.66 (s, 2H, CH_2), 6.82 [d, J=8.6 Hz, 2H, C(3)*H* and C(5)*H*] and 7.18 [d, J=8.6 Hz, 2H, C(2)*H* and C(6)*H*]; ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.9 (CH_2CN), 112.6 [*C*(3 and 5)], 120.2 (*C*N), 121.5 [*C*(1)], 128.9 [C(2 and 6)] and 151.0 [C(4)]; EI-MS *m/z* 147 [$(M)^+$, 65%], 131 (90) and 116 (100).

5'-Cyanomethyl-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-dihydroxyindolo-[2',3':6,7]morphinan (75)

4-Hydrazinobenzyl cyanide (1.30 g, 8.84 mmol) and naltrexone (**25**) (2.60 g, 7.62 mmol) were refluxed under N_2 for 3 h in EtOH/2N HCl (5:1). The solution was concentrated, dissolved in

H_2O, basified with NH_4OH and extracted with CH_2Cl_2. The combined organic extracts were concentrated, dried (Na_2SO_4), filtered and purified by column chromatography [$CH_3OH/CH_2Cl_2/NH_4OH$ (5:94.5:0.5)] affording 5'-cyanomethyl-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-dihydroxyindolo[2',3':6,7]morphinan (**75**) (2.83 g, 6.25 mmol, 82%) mp. 230 °C (dec.); R_f = 0.44 [$CH_3OH/CH_2Cl_2/NH_4OH$ (2:20:0.1)]; IR ν_{max}/cm ($CHCl_3$) 3466-3005 (br, OH) and 2250 (CN); ^1H NMR (300 MHz, $CDCl_3$) δ 0.18-0.23 [m, 2H, $NCH_2CH(CHHCHH)$], 0.57-0.65 [m, 2H, $NCH_2CH(CHHCHH)$], 0.89-0.97 [m, 1H, $NCH_2CH(CH_2CH_2)$], 5.62 [s, 1H, C(5)*H*], 6.54 [d, J=8.2 Hz, 1H, C(1)*H*], 6.62 [d, J=8.2 Hz, 1H, C(2)*H*], 6.97 [d, J=8.6 Hz, 1H, C(6')*H*], 7.23 [d, J=8.6 Hz, 1H, C(7')*H*] and 7.28-7.32 [m, 1H, C(4')*H*]; ^{13}C NMR (75 MHz, $CDCl_3$) δ 3.8 [$NCH_2CH(CH_2CH_2)$], 4.3 [$NCH_2CH(CH_2CH_2)$], 9.2 [$NCH_2CH(CH_2CH_2)$], 23.5 [C(10)], 24.9 (CH_2CN), 28.9 [C(15)], 31.4 [C(8)], 44.1 [C(16)], 49.9 [quaternary C(13)], 59.5 [C(18)], 62.5 [C(9)], 73.1 [quaternary C(14)], 84.9 [C(5)], 112.2 [C(7')], 117.4 [C(2)], 118.3 [C(4')], 119.1 (CN), 119.3 [C(1)], 120.2 (quaternary Ar), 122.3 [C(6')], 124.5 (quaternary Ar), 128.1 (quaternary Ar), 128.2 (quaternary Ar), 130.7 (quaternary Ar), 130.9 (quaternary Ar), 137.0 (quaternary Ar), 139.9 (quaternary Ar) and 143.5 (quaternary Ar); EI-MS *m/z* 453 [$(M)^+$, 20%], 412 (5) and 84 (100).

5'-Aminoethyl-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-dihydroxyindolo-[2',3':6,7]morphinan (76)

Method 1:

5'-Cyanomethyl-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-dihydroxyindolo[2',3':6,7]-morphinan (**75**) (1.02 g, 2.25 mmol) was dissolved in EtOH, and hydrogenated with Raney nickel at 25 psi for 72 h. The mixture was filtered through cellite, concentrated, dried (Na_2SO_4) and purified by column chromatography, [$CH_3OH/CH_2Cl_2/NH_4OH$ (10:89:1)] to give 5'-aminoethyl-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-dihydroxyindolo[2',3':6,7]-morphinan (**76**) (0.73 g, 1.60 mmol, 71%).

Method 2:

5'-Cyanomethyl-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-dihydroxyindolo[2',3':6,7]-morphinan (**75**) (0.53 g, 1.17 mmol) was dissolved in formic acid. Raney nickel catalyst was added and the mixture refluxed gently for 3 h. The mixture was then filtered through cellite and concentrated before being dissolved in water, basified with NH_4OH and extracted with [$CHCl_3$/isopropanol (7:3)]. The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Further purification was achieved by column chromatography, [$CH_3OH/CH_2Cl_2/NH_4OH$ (10:89:1)] giving 5'-aminoethyl-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-dihydroxyindolo[2',3':6,7]morphinan (**76**) (0.48 g, 1.04 mmol, 89%) mp. >250 °C (dec.); R_f = 0.17 [$CH_3OH/CH_2Cl_2/NH_4OH$ (10:89:1)]; HPLC (C_{18} column) 14.158 [MeOH/0.3% NH_4CO_3 (80:20)]; IR ν_{max}/cm ($CHCl_3$) 3572-3278 (br, NH_2 and OH); ^{1}H NMR (270 MHz, CD_3OD) δ 0.19-0.25 [m, 2H, $NCH_2CH(CHHCHH)$], 0.58-0.62 [m, 2H, $NCH_2CH(CHHCHH)$], 0.88-1.0 [m, 1H, $NCH_2CH(CH_2CH_2)$], 5.62 [s, 1H, C(5)*H*], 6.51-6.59 [m, 2H, C(1)*H* and C(2)*H*], 6.97 [d, J=8.2 Hz, 1H, C(6')*H*], 7.22-7.26 [m, 1H, *C*(4')] and 7.31 [d, J=8.2 Hz, 1H, C(7')*H*]; ^{13}C NMR (67.8 MHz, CD_3OD) δ 3.2 [$NCH_2CH(CH_2CH_2)$], 3.5 [$NCH_2CH(CH_2CH_2)$], 9.0 [$NCH_2CH(CH_2CH_2)$], 22.7 [*C*(10)], 28.3 [*C*(15)], 31.1 [*C*(8)], 38.3 ($CH_2CH_2NH_2$), 42.7 (CH_2NH_2), 43.3 [*C*(16)], 47.4 [quaternary *C*(13)], 59.1 [*C*(18)], 62.0 [*C*(9)], 72.6 [quaternary *C*(14)], 84.5 [*C*(5)], 109.7 (quaternary Ar), 111.0 [*C*(7')], 116.7 [*C*(2)], 118.1 [*C*(4')], 118.3 [*C*(1)], 123.0 [*C*(6')], 124.2 (quaternary Ar), 126.6 (quaternary Ar), 128.6 (quaternary Ar), 129.6 (quaternary Ar), 130.4 (quaternary Ar), 135.9 (quaternary Ar), 139.3 (quaternary Ar) and 143.0 (quaternary Ar); EI-MS *m/z* 457 [$(M)^+$, 60%] and 428 (100).

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-[(N-(3-methyl)butylamidino)ethyl]-3,14-dihydroxyindolo[2',3':6,7]morphinan (71)

Isovaleronitrile (1.19 g, 14.35 mmol) was dissolved in anhydrous EtOH. Dry HCl was bubbled through the solution for 40 min, after which the flask was sealed and left at -20 °C for 7 days.[74] The iminoether was crystallized from the resultant viscous solution, washed with ether and dried under a stream of N_2 for 2 h (0.71 g, 4.30 mmol, 30%).

The above iminoether (0.09 g, 0.55 mmol) and 5'-aminoethyl-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-dihydroxyindolo[2',3':6,7]morphinan (**76**) (0.10 g, 0.22 mmol) were stirred in anhydrous EtOH (5 ml) under N_2 for 72 h. Concentration and subsequent preparative thin layer chromatography [$CH_3OH/CH_2Cl_2/NH_4OH$ (15:84:1)] allowed isolation of branched amidine (**71**) (0.018 g, 0.03 mmol, 15.2%). The compound was converted to its dihydrochloride salt, mp. >250 °C (dec.); R_f = 0.21 [$CH_3OH/CH_2Cl_2/NH_4OH$ (20:80:1)]; HPLC (C_{18} column) 1.792 [MeOH/0.3% NH_4CO_3 (80:20)]; IR ν_{max}/cm (KBr) 3675-3008 (br, bonded OH and C=NH) and 1654 (C=N); ^{1}H NMR (270 MHz, CD_3OD) δ 0.42-0.50 [m, 2H, $NCH_2CH(CHHCHH)$], 0.58-0.69 [m, 2H, $NCH_2CH(CHHCHH)$], 0.82-0.88 [m, 1H, $NCH_2CH(CH_2CH_2)$], 5.62 [s, 1H, C(5)*H*], 6.55-6.61 [m, 2H, C(1)*H* and C(2)*H*], 6.94 [d, J=8.2 Hz, 1H, C(6')*H*], 7.21-7.24 [m, 1H, *C*(4')] and 7.25 [d, J=8.2 Hz, 1H, C(7')*H*]; ^{13}C NMR (75 MHz, CD_3OD) δ 2.2 [$NCH_2CH(CH_2CH_2)$], 4.9 [$NCH_2CH(CH_2CH_2)$], 5.7 [$NCH_2CH(CH_2CH_2)$], 20.8 [2xCH_3], 23.9 [*C*(10)], 27.2 [*C*(15)], 28.5 [*C*(8)], 29.0 (CH_2CH_2NH), 33.3 (CH_2NH), 40.7 [$C(NH)CH_2$], 41.4 [*C*(16)], 47.1 [quaternary *C*(13)], 57.8 [*C*(18)], 62.5 [*C*(9)], 72.5 [quaternary *C*(14)], 83.9 [*C*(5)], 108.2 (quaternary Ar), 111.7 [*C*(7')], 118.1 [*C*(2)], 118.4 and 119.3 [*C*(4')] and [*C*(1)], 121.5 (quaternary Ar), 123.1 [*C*(6')], 126.8 (quaternary Ar), 127.1 (quaternary Ar), 129.1 (quaternary Ar), 129.8 (quaternary Ar), 136.8 (quaternary Ar), 140.7 (quaternary Ar), and 143.6 (quaternary Ar); FAB-MS *m/z* 541 [$(M+1)^+$, 70%] and 458 (50); HRMS (EI) *m/z* 540.3104 $(M)^+$, $C_{33}H_{40}N_4O_3$ requires 540.3100.

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(thioethylimidic acid phenyl ester)-3,14-dihydroxyindolo[2',3':6,7]morphinan (78)

A solution of 5'-cyanomethyl-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-dihydroxy-indolo[2',3':6,7]morphinan (**75**) (0.200 g, 0.44 mmol), thiophenol (0.050 g, 0.046 ml, 0.44 mmol) and anhydrous MeOH (100 ml) in an ice-salt bath, was purged with dry nitrogen. HBr was bubbled through the solution for 45 min. The solution was partially concentrated *in vacuo* and Et_2O was added. The Et_2O was decanted, removing excess unreacted thiophenol. Et_2O was again added and the crude thioimidic ester precipitated out. Since the crude compound was not particularly stable, it was used in the following reactions without further purification.

5'-(N-propyl)acetamidino-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-dihydroxyindolo-[2',3':6,7]morphinan (72)

Propylamine (0.010 g, 0.014 ml, 0.175 mmol) and thioimidic ether (**78**) (0.129 g, 0.229 mmol) in anhydrous MeOH (25ml) were reacted according to general procedure B, to give major product (**72**) (0.024 g, 0.047 mmol, 27%). The dihydrobromide salt of the product was then formed, mp. 249 °C (dec.); R_f = 0.22 [$CH_3OH/CH_2Cl_2/NH_4OH$ (20:80:1)]; HPLC (C_{18} column) 2.696 [MeOH/0.3% NH_4CO_3 (80:20)]; IR ν_{max}/cm (KBr) 3680-2700 (br, bonded OH and C=NH) and 1670 (C=N); ^{1}H NMR (270 MHz, CD_3OD) δ 0.35-0.42 [m, 2H, $NCH_2CH(CHHCHH)$], 0.62-0.76 [m, 2H, $NCH_2CH(CHHCHH)$], 0.87-0.93 [m, 1H, $NCH_2CH(CH_2CH_2)$], 5.65 [s, 1H, C(5)*H*], 6.61-6.63 [m, 2H, C(1)*H* and C(2)*H*], 7.10 [d, J=8.6 Hz, 1H, C(6')*H*], 7.35 [d, J=8.6 Hz, 1H, C(7')*H*] and 7.44-7.47 [m, 1H, *C*(4')]; ^{13}C NMR (75 MHz, CD_3OD) δ 3.8 [$NCH_2CH(CH_2CH_2)$], 5.7 [$NCH_2CH(CH_2CH_2)$], 8.3 [$NCH_2CH(CH_2CH_2)$], 11.5 (CH_3), 22.0 [*C*(10)], 24.8 ($NHCH_2CH_2$), 30.2 [*C*(15)], 30.9 [*C*(8)], 40.1 ($NHCH_2$) 45.3 [*C*(16)], 46.5 (CH_2C=NH), 48.7 [quaternary *C*(13)], 59.5 [*C*(18)], 63.6 [*C*(9)], 74.0 [quaternary *C*(14)], 85.4 [*C*(5)], 110.4 (quaternary Ar), 113.1 [*C*(7')], 119.0 [*C*(2)], 120.4 [*C*(1)] and [*C*(4')], 124.0 [*C*(6')], 124.6 (quaternary Ar), 128.4 (quaternary Ar), 131.0 (quaternary Ar), 131.8 (quaternary Ar), 138.4 (quaternary Ar), 141.6 (quaternary Ar), 144.8 (quaternary Ar) and 168.7 (*C*=NH); EI-MS *m/z* 512 [$(M)^+$, 100%], 453 (80) and 412 (25); HRMS (EI) *m/z* 512.2788 $(M)^+$, $C_{31}H_{36}N_4O_3$ requires 512.2787. Minor product (**79**) EI-MS *m/z* 554 [$(M)^+$, 100%], 453 (40) and 428 (65).

5'-(N-pentyl)acetamidino-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-dihydroxyindolo-[2',3':6,7]morphinan (73)

Amylamine (0.011 g, 0.015 ml, 0.125 mmol) and thioimidic ether (**78**) (0.090 g, 0.159 mmol) in anhydrous MeOH (25ml) were reacted according to general procedure B, to give major product (**73**) (0.016 g, 0.030 mmol, 24%). The dihydrobromide salt of the product was then formed, mp. 240-243 °C (dec.); R_f = 0.31 [$CH_3OH/CH_2Cl_2/NH_4OH$ (20:80:1)]; HPLC (C_{18} column) 3.017 [MeOH/0.3% NH_4CO_3 (80:20)]; IR ν_{max}/cm (KBr) 3620-2800 (br, bonded OH and C=NH) and 1670 (C=N); ^{1}H NMR (270 MHz, CD_3OD) δ 0.36-0.49 [m, 2H, $NCH_2CH(CHHCHH)$], 0.67-0.84

[m, 2H, $NCH_2CH(CHHCHH)$], 0.88-0.98 [m, 1H, $NCH_2CH(CH_2CH_2)$], 5.67 [br s, 1H, C(5)*H*], 6.54-6.66 [m, 2H, C(1)*H* and C(2)*H*], 6.99-7.12 [m, 1H, *C*(4')] and 7.25-7.44 [m, 2H, C(6')*H* and C(7')*H*]; ^{13}C NMR (67.8 MHz, CD_3OD) δ 2.4 [$NCH_2CH(CH_2CH_2)$], 4.5 [$NCH_2CH(CH_2CH_2)$], 6.5 [$NCH_2CH(CH_2CH_2)$], 12.7 (CH_3), 21.9 [*C*(10)], 26.9, 27.0, 28.2 ($NHCH_2CH_2CH_2CH_2CH_3$), 28.6 [*C*(15)], 30.4 [*C*(8)], 39.5 ($NHCH_2$) 42.4 [*C*(16)], 45.6 ($CH_2C{=}NH$), 48.0 [quaternary *C*(13)], 58.1 [*C*(18)], 62.4 [*C*(9)], 72.6 [quaternary *C*(14)], 84.0 [*C*(5)], 108.9 (quaternary Ar), 111.8 [*C*(7')], 117.9 (Ar), 119.0 (Ar), 119.1 (Ar), 122.8 (Ar), 123.3 (Ar), 127.1 (Ar), 129.5 (quaternary Ar), 130.5 (quaternary Ar), 137.2 (quaternary Ar), 140.5 (quaternary Ar), 143.5 (quaternary Ar) and 167.4 (*C*=NH); EI-MS *m/z* 540 [$(M)^+$, 100%], 453 (50) and 412 (20); HRMS (EI) *m/z* 540.3082 $(M)^+$, $C_{33}H_{40}N_4O_3$ requires 540.3100. Minor product (**80**) EI-MS *m/z* 610 [$(M)^+$, 15%], 471 (5), 453 (2) and 428 (8).

5'-(N-heptyl)acetamidino-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-dihydroxyindolo-[2',3':6,7]morphinan (74)

Heptylamine (0.014 g, 0.018 ml, 0.122 mmol) and thioimidic ether (**78**) (0.088 g, 0.156 mmol) in anhydrous MeOH (25ml) were reacted according to general procedure B, to give major product (**74**) (0.016 g, 0.028 mmol, 23%). The dihydrobromide salt of the product was then formed, mp. 220 °C (dec.); R_f = 0.47 [$CH_3OH/CH_2Cl_2/NH_4OH$ (20:80:1)]; HPLC (C_{18} column) 4.475 [MeOH/0.3% NH_4CO_3 (80:20)]; IR ν_{max}/cm (KBr) 3640-2840 (br, bonded OH and C=NH) and 1670 (C=N); ^1H NMR (270 MHz, CD_3OD) δ 0.28-0.37 [m, 2H, $NCH_2CH(CHHCHH)$], 0.72-0.78 [m, 2H, $NCH_2CH(CHHCHH)$], 0.79-0.86 [m, 1H, $NCH_2CH(CH_2CH_2)$], 5.58 [s, 1H, C(5)*H*], 6.51-6.55 [m, 2H, C(1)*H* and C(2)*H*], 6.99 [d, J=8.6 Hz, 1H, C(6')*H*], 7.27 [d, J=8.6 Hz, 1H, C(7')*H*] and 7.32-7.35 [m, 1H, *C*(4')]; ^{13}C NMR (67.8 MHz, CD_3OD) δ 2.6 [$NCH_2CH(CH_2CH_2)$], 3.9 [$NCH_2CH(CH_2CH_2)$], 7.7 [$NCH_2CH(CH_2CH_2)$], 13.0 (CH_3), 22.1 [*C*(10)], 23.2, 26.3, 27.3, 28.4 and 28.7 ($NHCH_2CH_2CH_2CH_2CH_2CH_2CH_3$), 30.4 [*C*(15)], 31.4 [*C*(8)], 42.3 ($NHCH_2$), 44.6 [*C*(16)], 46.7 ($CH_2C{=}NH$), 48.1 [quaternary *C*(13)], 58.7 [*C*(18)], 62.4 [*C*(9)], 72.9 [quaternary *C*(14)], 84.3 [*C*(5)], 109.5 (quaternary Ar), 111.8 [*C*(7')], 117.5 (Ar), 118.7 (Ar), 118.9 (Ar), 122.6 (Ar), 123.1 (Ar), 127.2 (Ar), 130.2 (quaternary Ar), 130.7 (quaternary Ar), 137.1 (quaternary Ar), 140.1 (quaternary Ar), 143.5 (quaternary Ar) and 167.4 (*C*=NH); EI-MS *m/z* 568 [$(M)^+$, 40%],

453 (100) and 412 (35); HRMS (EI) *m/z* 568.3403 $(M)^+$, $C_{35}H_{44}N_4O_3$ requires 568.3413. Minor product (**81**) EI-MS *m/z* 666 [$(M)^+$, 45%], 453 (20) and 428 (20).

±17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-[4-methyl-(4,5-dihydro-1H-imidazol-2-yl)methyl]-3,14-dihydroxyindolo[2',3':6,7]morphinan (83)

1,2-Diaminopropane.*p*-TSA (2.19 g, 8.91 mmol) and 5'-cyanomethyl-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-dihydroxyindolo[2',3':6,7]morphinan (**75**) (0.41 g, 0.90 mmol) were reacted according to general procedure C, giving methyl imidazoline (**83**) (0.025 g, 0.048 mmol, 5%). The product was converted to the dihydrochloride salt, mp. 191-193 °C; R_f = 0.37 [$CH_3OH/CH_2Cl_2/NH_4OH$ (20:80:1)]; HPLC (C_{18} column) 2.575 [MeOH/0.3% NH_4CO_3 (80:20)]; IR ν_{max}/cm (KBr) 3660-2700 (br, bonded OH and NH); ^{1}H NMR (270 MHz, CD_3OD) δ 0.14-0.26 [m, 2H, $NCH_2CH(CHHCHH)$], 0.50-0.61 [m, 2H, $NCH_2CH(CHHCHH)$], 0.85-0.98 [m, 1H, $NCH_2CH(CH_2CH_2)$], 5.57 [s, 1H, C(5)*H*], 6.51-6.55 [m, 2H, C(1)*H* and C(2)*H*], 6.97-7.04 [m, 1H, Ar*H*] and 7.26-7.35 [m, 2H, 2x Ar*H*]; ^{13}C NMR (67.8 MHz, CD_3OD) δ 2.8 [$NCH_2CH(CH_2CH_2)$], 3.3 [$NCH_2CH(CH_2CH_2)$], 8.9 [$NCH_2CH(CH_2CH_2)$], 19.8 (CH_3), 20.1 (CH_2), 22.8 (CH_2), 28.5 (CH_2), 31.4 (CH_2), 43.6 (CH_2), 46.7 (CH_2), 46.9 (CH_2), 47.2 [quaternary *C*(13)], 52.7 (*C*H), 59.2 [*C*(18)], 62.3 [*C*(9)], 73.1 [quaternary *C*(14)], 84.6 [*C*(5)], 110.0 (Ar), 111.6 (Ar), 117.1 (Ar), 118.4 (Ar), 118.8 (Ar), 122.6 (Ar), 124.7 (Ar), 127.2 (Ar), 128.6 (Ar), 130.8 (Ar), 137.0 (Ar), 139.9 (Ar) and 143.4 (Ar); EI-MS *m/z* 510 [$(M)^+$, 100%] and 455 (20); HRMS (EI) *m/z* 510.2634 $(M)^+$, $C_{31}H_{34}N_4O_3$ requires 510.2631.

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-[4,4-dimethyl-(4,5-dihydro-1H-imidazol-2-yl)methyl]-3,14-dihydroxyindolo[2',3':6,7]morphinan (84)

1,2-Diamino-2-methylpropane.*p*-TSA (2.14 g, 8.22 mmol) and 5'-cyanomethyl-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-dihydroxyindolo[2',3':6,7]morphinan (**75**) (0.38 g, 0.83 mmol) were reacted according to general procedure C, giving dimethyl imidazoline (**84**) (0.028 g, 0.053 mmol, 6%). The product was converted to the dihydrochloride salt, mp. >220 °C; R_f = 0.40 [$CH_3OH/CH_2Cl_2/NH_4OH$ (20:80:1)]; IR ν_{max}/cm (KBr) 3640-2580 (br, bonded

OH and NH); ^{1}H NMR (270 MHz, CD_3OD) δ 0.12-0.25 [m, 2H, $NCH_2CH(CHHCHH)$], 0.48-0.63 [m, 2H, $NCH_2CH(CHHCHH)$], 0.86-0.99 [m, 1H, $NCH_2CH(CH_2CH_2)$], 5.52 [s, 1H, C(5)*H*], 6.45-6.53 [m, 2H, C(1)*H* and C(2)*H*], 7.09-7.16 [m, 1H, Ar*H*] and 7.21-7.30 [m, 2H, 2x Ar*H*]; ^{13}C NMR (67.8 MHz, CD_3OD) δ 2.8 [$NCH_2CH(CH_2CH_2)$], 3.3 [$NCH_2CH(CH_2CH_2)$], 9.0 [$NCH_2CH(CH_2CH_2)$], 19.0 (CH_3), 22.4 (CH_3), 23.9 (CH_2), 27.1 (CH_2), 27.5 (CH_2), 28.5 (CH_2), 29.4 (CH_2), 31.5 (CH_2), 43.7 (CH_2), 47.8 [quaternary *C*(13)], 59.2 [*C*(18)], 62.4 [*C*(9)], 73.2 [quaternary *C*(14)], 84.3 [*C*(5)], 109.8 (Ar), 111.2 (Ar), 117.7 (Ar), 118.3 (Ar), 118.4 (Ar), 122.6 (Ar), 127.1 (Ar), 130.5 (Ar), 130.6 (Ar), 136.7 (quaternary Ar) and 143.9 (quaternary Ar); EI-MS *m/z* 524 [$(M)^+$, 100%] and 469 (15); HRMS (EI) *m/z* 524.2784 $(M)^+$, $C_{32}H_{36}N_4O_3$ requires 524.2787.

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N-ethyl-N'-ethylurea)-3,14-dihydroxyindolo[2',3':6,7]morphinan (85)

5'-Aminoethyl-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-dihydroxyindolo[2',3':6,7]-morphinan (**76**) (0.100 g, 0.220 mmol) and ethyl isocyanate (0.016 g, 0.017 ml, 0.225 mmol) were reacted according to general procedure D, yielding (**85**) (0.035 g, 0.067 mmol, 30%), which was subsequently converted to the dihydrochloride salt, mp. 203-206 °C (dec); R_f = 0.56 [$CH_3OH/CH_2Cl_2/NH_4OH$ (10:89:1)]; HPLC (C_{18} column) 1.892 [MeOH/0.3% NH_4CO_3 (80:20)]; IR ν_{max}/cm (KBr) 3660-2600 (br, bonded OH and CONH) and 1630 (urea); ^{1}H NMR (400 MHz, CD_3OD) δ 0.49-0.59 [m, 2H, $NCH_2CH(CHHCHH)$], 0.74-0.82 [m, 1H, $NCH_2CH(CH_2CH_2)$], 0.84-0.91 [m, 2H, $NCH_2CH(CHHCHH)$], 5.70 [s, 1H, C(5)*H*], 6.65 [d, J=8.2, 1H, C(1)*H*], 6.67 [d, J=8.2 Hz, 1H, C(2)*H*], 7.00 [d, J=8.2 Hz, 1H, C(6')*H*], 7.25 [s, 1H, C(4')*H*] and 7.29 [d, J=8.2 Hz, 1H, C(7')*H*]; ^{13}C NMR (100 MHz, CD_3OD) δ 3.5 [$NCH_2CH(CH_2CH_2)$], 6.4 [$NCH_2CH(CH_2CH_2)$], 7.0 [$NCH_2CH(CH_2CH_2)$], 15.4 (CH_3), 25.1 [*C*(10)], 29.9 [*C*(15)], 30.4 [*C*(8)], 36.3 (CH_2), 36.5 (CH_2), 37.4 (CH_2), 43.7 [*C*(16)], 47.6 [quaternary *C*(13)], 58.9 [*C*(18)], 63.7 [*C*(9)], 73.6 [quaternary *C*(14)], 85.2 [*C*(5)], 109.1 (Ar), 112.3 (Ar), 119.3 (Ar), 120.3 (Ar), 122.4 (Ar), 124.7 (Ar), 128.0 (Ar), 130.3 (Ar), 130.5 (Ar), 137.7 (Ar), 141.9 (Ar), 144.7 (Ar) and 161.6 (C=O); FAB-MS *m/z* 529 [$(M+1)^+$, 100%] and 330 (22); HRMS (FAB) *m/z* 529.2838 $(M+1)^+$, $C_{31}H_{37}N_4O_4$ requires 529.2815. The minor product (**89**) was also isolated (0.010 g, 0.017 mmol,

8%) R_f = 0.68 [$CH_3OH/CH_2Cl_2/NH_4OH$ (10:89:1)]; IR ν_{max}/cm (KBr) 3640-2700 (br, bonded OH and CONH) and 1630 (urea); ^1H NMR (400 MHz, CD_3OD) δ 0.52-0.59 [m, 2H, $NCH_2CH(CHHCHH)$], 0.75-0.82 [m, 1H, $NCH_2CH(CH_2CH_2)$], 0.85-0.93 [m, 2H, $NCH_2CH(CHHCHH)$], 5.77 [s, 1H, C(5)*H*], 6.80 [d, J=8.4, 1H, C(1)*H*], 6.87 [d, J=8.4 Hz, 1H, C(2)*H*], 7.01 [d, J=8.2 Hz, 1H, C(6')*H*], 7.24 [s, 1H, C(4')*H*] and 7.26 [d, J=8.2 Hz, 1H, C(7')*H*]; FAB-MS *m/z* 600 [$(M+1)^+$, 100%], 529 (20) and 413 (30).

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N-ethyl-N'-butylurea)-3,14-dihydroxyindolo[2',3':6,7]morphinan (86)

5'-Aminoethyl-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-dihydroxyindolo[2',3':6,7]-morphinan (**76**) (0.102 g, 0.223 mmol) and butyl isocyanate (0.022 g, 0.025 ml, 0.222 mmol) were reacted according to general procedure D, yielding (**86**) (0.041 g, 0.073 mmol, 33%). The product was then converted to the dihydrochloride salt, mp. 179-181 °C (dec); R_f = 0.42 [$CH_3OH/CH_2Cl_2/NH_4OH$ (10:89:1)]; HPLC (C_{18} column) 1.967 [MeOH/0.3% NH_4CO_3 (80:20)]; IR ν_{max}/cm (KBr) 3660-2500 (br, bonded OH and CONH) and 1630 (urea); ^1H NMR (400 MHz, CD_3OD) δ 0.47-0.59 [m, 2H, $NCH_2CH(CHHCHH)$], 0.71-0.80 [m, 1H, $NCH_2CH(CH_2CH_2)$], 5.69 [s, 1H, C(5)*H*], 6.65-6.71 [m, 2H, C(1)*H* and C(2)*H*], 7.00 [d, J=8.2 Hz, 1H, C(6')*H*], 7.25 [s, 1H, C(4')*H*] and 7.29 [d, J=8.2 Hz, 1H, C(7')*H*]; ^{13}C NMR (100 MHz, CD_3OD) δ 3.4 [$NCH_2CH(CH_2CH_2)$], 6.3 [$NCH_2CH(CH_2CH_2)$], 6.9 [$NCH_2CH(CH_2CH_2)$], 14.0 (CH_3), 20.8 (CH_2), 25.1 [*C*(10)], 29.8 [*C*(15)], 30.3 [*C*(8)], 32.7 (CH_2), 35.3 (CH_2), 37.1 (CH_2), 41.3 (CH_2), 43.8 [*C*(16)], 47.5 [quaternary *C*(13)], 58.8 [*C*(18)], 64.0 [*C*(9)], 73.7 [quaternary *C*(14)], 85.2 [*C*(5)], 109.3 (Ar), 112.5 (Ar), 119.4 (Ar), 119.5 (Ar), 120.6 (Ar), 122.6 (Ar), 124.8 (Ar), 128.1 (Ar), 130.4 (Ar), 130.7 (Ar), 135.4 (Ar), 137.8 (Ar), 142.0 (Ar) and 144.8 (Ar); FAB-MS *m/z* 557 [$(M+1)^+$, 100%] and 371 (10); HRMS (FAB) *m/z* 557.3132 $(M+1)^+$, $C_{33}H_{41}N_4O_4$ requires 557.3128.

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N-ethyl-N'-hexylurea)-3,14-dihydroxyindolo[2',3':6,7]morphinan (87)

5'-Aminoethyl-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-dihydroxyindolo[2',3':6,7]-morphinan (**76**) (0.100 g, 0.219 mmol) and hexyl isocyanate (0.028 g, 0.032 ml, 0.220 mmol) were reacted according to general procedure D, yielding (**87**) (0.052 g, 0.089 mmol, 40%), which was then converted to the dihydrochloride salt, mp. 240 °C (dec); R_f = 0.37 [$CH_3OH/CH_2Cl_2/NH_4OH$ (10:89:1)]; HPLC (C_{18} column) 1.800 [MeOH/0.3% NH_4CO_3 (80:20)]; IR ν_{max}/cm (KBr) 3640-2680 (br, bonded OH and CONH) and 1630 (urea); ^{1}H NMR (270 MHz, CD_3OD) δ 0.49-0.60 [m, 2H, $NCH_2CH(CHHCHH)$], 0.72-0.81 [m, 1H, $NCH_2CH(CH_2CH_2)$], 5.70 [s, 1H, C(5)*H*], 6.62-6.71 [m, 2H, C(1)*H* and C(2)*H*], 7.00 [d, J=8.2 Hz, 1H, C(6')*H*], 7.26 [s, 1H, C(4')*H*] and 7.29 [d, J=8.2 Hz, 1H, C(7')*H*]; ^{13}C NMR (67.8 MHz, CD_3OD) δ 2.2 [$NCH_2CH(CH_2CH_2)$], 5.0 [$NCH_2CH(CH_2CH_2)$], 5.6 [$NCH_2CH(CH_2CH_2)$], 13.0 (CH_3), 22.2 (CH_2), 23.9 [*C*(10)], 26.1 (CH_2), 28.6 [*C*(15)], 29.1 [*C*(8)], 29.2 (CH_2), 31.2 (CH_2), 35.8 (CH_2), 40.3 (CH_2), 42.6 [*C*(16)], 46.7 [quaternary *C*(13)], 57.7 [*C*(18)], 62.6 [*C*(9)], 72.5 [quaternary *C*(14)], 84.0 [*C*(5)], 108.0 (quaternary Ar), 111.2 (Ar), 118.1 (Ar), 118.2 (Ar), 119.2 (Ar), 121.3 (quaternary Ar), 123.6 (Ar), 126.9 (quaternary Ar), 129.1 (quaternary Ar), 129.5 (quaternary Ar), 136.6 (quaternary Ar), 140.8 (quaternary Ar) and 143.6 (quaternary Ar); FAB-MS *m/z* 585 [$(M+1)^+$, 100%] and 326 (10); HRMS (FAB) *m/z* 585.3414 $(M+1)^+$, $C_{35}H_{45}N_4O_4$ requires 585.3441.

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N-ethyl-N'-ethylurea)-3,14-dihydroxyindolo[2',3':6,7]morphinan (85)

Carbamate (**89**) (0.010 g, 0.017 mmol) was stirred in [CH_3OH/H_2O (9:1)] with K_2CO_3 (5 eq) for 12 h. The CH_3OH was removed *in vacuo*, the aqueous phase extracted with CH_2Cl_2 and subsequently purified by preparative thin layer chromatography [^{i}PrOH/$CHCl_3$ (3:7)], yielding ethyl urea (**85**) (0.003 g, 0.006 mmol, 36%). Characterisation as above.

5'-Cyanomethyl-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-14-hydroxy-3-trityloxy-indolo[2',3':6,7]morphinan (95)

5'-Cyanomethyl-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-di-hydroxyindolo-[2',3':6,7]morphinan (**75**) (2.22 g, 4.91 mmol), triphenylmethyl chloride (1.53 g, 5.48 mmol), triethylamine (0.84 g, 1.15 ml, 8.32 mmol) and a catalytic amount of 4-dimethylaminopyridine (DMAP) (0.060g) were added to anhydrous CH_2Cl_2 (50 ml) and stirred at RT, under N_2, for 12 h. The solution was then basified with $NaHCO_3$ and extracted with CH_2Cl_2. The combined organic extracts were dried (Na_2SO_4), concentrated, filtered and purified by column chromatography [$CH_3OH/CH_2Cl_2/NH_4OH$ (2:97:1)] affording 5'-cyanomethyl-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-14-hydroxy-3-trityloxyindolo[2',3':6,7]morphinan (**95**) (2.22 g, 3.19 mmol, 65%); mp. 156-157 °C; R_f = 0.50 [$CH_3OH/CH_2Cl_2/NH_4OH$ (2:97:1)]; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.35-0.46 [m, 2H, $NCH_2CH(CHHCHH)$], 0.76-0.82 [m, 2H, $NCH_2CH(CHHCHH)$], 1.06-1.18 [m, 1H, $NCH_2CH(CH_2CH_2)$], 5.60 [s, 1H, C(5)*H*], 6.41-6.58 [m, 2H, C(1)*H* and C(2)*H*], 7.00-7.07 (m, 1H, Ar*H*), 7.12-7.18 (m, 1H, Ar*H*) and 7.24-7.60 (m, 16H, Ar*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 3.9 [$NCH_2CH(CH_2CH_2)$], 4.1 [$NCH_2CH(CH_2CH_2)$], 9.5 [$NCH_2CH(CH_2CH_2)$], 23.3 [C(10)], 23.7 (CH_2CN), 28.7 [C(15)], 31.1 [C(8)], 43.4 [C(16)], 47.6 [quaternary C(13)], 59.4 [C(18)], 62.0 [C(9)], 72.2 [quaternary C(14)], 84.0 [C(5)], 92.0 [$C(CH_3)$], 110.9 (Ar), 111.6 (Ar), 117.7 (Ar), 117.9 (Ar), 118.8 (Ar), 120.0 (CN), 121.9 (Ar), 124.9 (Ar), 126.8 (Ar), 127.0 (4 x Ar), 127.6 (Ar), 127.7 (2 x Ar), 127.7 (2 x Ar), 128.4 (Ar), 128.5 (Ar), 129.2 (Ar), 130.1 (Ar), 130.5 (Ar), 136.3 (Ar), 138.6 (Ar), 143.9 (Ar), 146.7 (Ar) and 149.4 (Ar); FAB-MS *m/z* 696 [$(M+1)^+$, 100%]; HRMS (FAB) *m/z* 696.3215 $(M+1)^+$, $C_{47}H_{42}N_3O_3$ requires 696.3226.

5'-Aminoethyl-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-14-hydroxy-3-trityloxy-indolo[2',3':6,7]morphinan (94)

5'-Cyanomethyl-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-14-hydroxy-3-tritylindolo-[2',3':6,7]morphinan (**95**) (2.07 g, 2.98 mmol) was dissolved in CH_3OH (50 ml), to which cyclohexene (50 ml) and a spatula tip of Raney nickel was added. The mixture was then stirred at *ca.* 50 °C for 6 h, before being dried (Na_2SO_4), concentrated, filtered and purified by column

chromatography [$CH_3OH/CH_2Cl_2/NH_4OH$ (10:89:1)] to give 5'-aminoethyl-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-14-hydroxy-3-trityloxyindolo[2',3':6,7]morphinan (**94**) (1.71 g, 2.44 mmol, 82%); mp. 228 °C; R_f = 0.31 [$CH_3OH/CH_2Cl_2/NH_4OH$ (10:89:1)]; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.32-0.41 [m, 2H, $NCH_2CH(CHHCHH)$], 0.73-0.84 [m, 2H, $NCH_2CH(CHHCHH)$], 0.89-1.01 [m, 1H, $NCH_2CH(CH_2CH_2)$], 5.60 [s, 1H, C(5)*H*], 6.36 [d, J=8.3Hz, 1H, C(1)*H*], 6.43 [d, J=8.3Hz, 1H, C(2)*H*] and 7.17-7.49 (m, 18H, Ar*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 3.7 [$NCH_2CH(CH_2CH_2)$], 3.9 [$NCH_2CH(CH_2CH_2)$], 9.3 [$NCH_2CH(CH_2CH_2)$], 23.1 [*C*(10)], 24.1 (CH_2), 25.4 (CH_2), 28.6 [*C*(15)], 31.0 [*C*(8)], 43.4 [*C*(16)], 47.5 [quaternary *C*(13)], 59.4 [*C*(18)], 62.0 [*C*(9)], 72.2 [quaternary *C*(14)], 84.3 [*C*(5)], 91.7 [$C(CH_3)$], 110.6 (quaternary Ar), 111.2 (Ar), 117.6 (Ar), 118.5 (Ar), 123.6 (Ar), 124.8 (Ar), 126.7 (Ar), 127.0 (Ar), 128.2 (quaternary Ar), 129.1 (Ar), 129.5 (quaternary Ar), 130.0 (quaternary Ar), 130.1 (quaternary Ar), 130.6 (quaternary Ar), 136.0 (quaternary Ar), 138.7 (quaternary Ar), 144.1 (quaternary Ar) and 149.5 (quaternary Ar); FAB^+MS *m/z* 700 [$(M+1)^+$, 18%] and 243 (100); HRMS (FAB) *m/z* 700.3572 $(M+1)^+$, $C_{47}H_{46}N_3O_3$ requires 700.3539.

Toluene-4-sulfonic acid 3-*tert*-butoxycarbonylamino propyl ester (96)[121]

3-Aminopropan-1-ol (3.00g, 40.0 mmol) was dissolved in tetrahydrofuran (THF) (20 ml) and stirred at 0 °C. $(BOC)_2O$ in THF (40 ml) was added dropwise and the reaction mixture allowed to warm to RT and stirred for 12 h. The solvent was then evaporated under reduced pressure and the residue purified by column chromatography R_f = 0.57 [$CH_3OH/CH_2Cl_2/NH_4OH$ (10:89:1)] to give 3-(N-BOC-amino)propan-1-ol (4.90 g, 28.0 mmol, 70%).

3-(N-BOC-amino)propan-1-ol (1.83 g, 10.46 mmol), 4-dimethylaminopyridine (1.28 g, 10.46 mmol) and triethylamine (1.60g, 2.20 ml, 15.70 mmol) were dissolved in anhydrous CH_2Cl_2 (50 ml) at 0 °C. *p*-Toluenesulfonyl chloride (4.47 g, 23.55 mmol) was dissolved in anhydrous CH_2Cl_2 (50 ml) and added dropwise to the reaction mixture over 1 h. The mixture was then stirred for 12 h at RT and subsequently washed with water. The organic layers were dried (Na_2SO_4), concentrated and further purified by column chromatography [Hexane/EtOAc (50:50)], to give toluene-4-sulfonic acid 3-*tert*-butoxycarbonylamino propyl ester (**96**) (1.20 g,

3.65 mmol, 35%) as a volatile, colourless liquid. R_f = 0.45 [Hexane/EtOAc (50:50)]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.32-1.48 (m, 9H, 3 x C*H*$_3$), 1.75-1.85 (m, 2H, C*H*$_2$), 2.40 (s, 3H, C*H*$_3$), 3.06-3.13 (m, 2H, C*H*$_2$), 4.00-4.09 (m, 2H, C*H*$_2$), 4.74 (br s, 1H, N*H*), 7.30 (d, J=8.6 Hz, 2H, Ar*H*) and 7.73 (d, J=8.6 Hz, 2H, Ar*H*). No characterisation reported in reference article.

5'-(Dimethylamino)ethyl-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-dihydroxy-indolo[2',3':6,7]morphinan (98)

Formaldehyde (0.017 g, 0.016 ml 37 WT.% solution, 0.21 mmol) was added to a solution of 5'-aminoethyl-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-dihydroxyindolo[2',3':6,7]-morphinan (**76**) (0.101 g, 0.22 mmol) in CH_3OH (5 ml) and stirred at RT for 30 min (the resulting imine precipitated from solution), after which acetic acid (0.024 ml) was added (which made the precipitate redissolve), followed by sodium cyanoborohydride (0.021g, 0.33 mmol). The reaction was then stirred at RT, under N_2, for 24 h. After this, sodium cyanoborohydride (0.021g, 0.33 mmol) and formaldehyde (0.016 ml, 1M) were added and the reaction stirred for a further 1 h, before being concentrated *in vacuo* and purified by column chromatography [$CH_3OH/CH_2Cl_2/NH_4OH$ (10:89:1)], to give 5'-(dimethylamino)ethyl-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-dihydroxyindolo[2',3':6,7]morphinan (**98**) (0.071 g, 0.15 mmol, 66%). The product was then converted to the dihydrochloride salt, mp. >225 °C; R_f = 0.26 [$CH_3OH/CH_2Cl_2/NH_4OH$ (10:89:1)]; ^{1}H NMR (400 MHz, CD_3OD) δ 0.47-0.62 [m, 2H, $NCH_2CH(CHHCHH)$], 0.72-0.94 [m, 2H, $NCH_2CH(CHHCHH)$], 1.08-1.21 [m, 1H, $NCH_2CH(CH_2CH_2)$], 5.69 [s, 1H, C(5)*H*], 6.61-6.69 [m, 2H, C(1)*H* and C(2)*H*], 7.00-7.07 (m, 1H, Ar*H*) and 7.32-7.39 [m, 2H, Ar*H*]; ^{13}C NMR (100 MHz, CD_3OD) δ 3.6 [$NCH_2CH(CH_2CH_2)$], 6.4 [$NCH_2CH(CH_2CH_2)$], 7.0 [$NCH_2CH(CH_2CH_2)$], 25.1 [*C*(10)], 29.8 [*C*(15)], 30.4 [*C*(8)], 32.0 (*C*H$_2$), 43.5 (*C*H$_2$), 43.6 (*C*H$_2$), 47.5 [quaternary *C*(13)], 48.0 (2 x *C*H$_3$) 58.9 [*C*(18)], 63.6 [*C*(9)], 73.7 [quaternary *C*(14)], 85.0 [*C*(5)], 109.4 (Ar), 112.9 (Ar), 119.2 (Ar), 119.7 (Ar), 120.5 (Ar), 122.5 (Ar), 124.3 (Ar), 127.4 (Ar), 128.2 (Ar), 130.2 (Ar), 131.0 (Ar), 137.9 (Ar), 141.7 (Ar) and 144.6 (Ar); FAB$^+$MS *m/z* 486 [(M+1)$^+$, 100%]; HRMS (FAB) *m/z* 486.2760 (M+1)$^+$, $C_{30}H_{36}N_3O_3$ requires 486.2757; Anal. ($C_{30}H_{35}N_3O_3$.2HCl.2H_2O) requires C 60.60, H 6.95, N 7.07, found C 60.7, H 6.92, N 6.98.

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N-ethyl)amido-3,14-dihydroxyindolo-[2',3':6,7]morphinan (99)

5'-Carboxy-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-dihydroxyindolo[2',3':6,7]-morphinan (**64**) (0.099 g, 0.200 mmol) and ethylamine hydrochloride (0.025 g, 0.303 mmol) were reacted according to general procedure A, to yield 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N-ethyl)amido-3,14-dihydroxyindolo[2',3':6,7]morphinan (**99**) (0.019 g, 0.040 mmol, 20%). The product was then converted to the hydrochloride salt, mp. >220 °C (dec); R_f = 0.28 [$CH_3OH/CH_2Cl_2/NH_4OH$ (10:89:1)]; IR ν_{max}/cm (KBr) 3640-2860 (br, bonded OH and amide NH), 1634 (amide I) and 1540 (amide II); ^{1}H NMR (400 MHz, CD_3OD) δ 0.50-0.69 [m, 2H, $NCH_2CH(CHHCHH)$], 0.74-0.81 [m, 1H, $NCH_2CH(CH_2CH_2)$], 0.84-0.93 [m, 2H, $NCH_2CH(CHHCHH)$], 5.71 [s, 1H, C(5)*H*], 6.67 [d, J=8.2 Hz, 1H, C(1)*H*], 6.70 [d, J=8.2 Hz, 1H, C(2)*H*], 7.41 [d, J=8.6 Hz, 1H, C(7')*H*], 7.60 [dd, J_1=8.6 Hz, J_2=1.5 Hz, 1H, C(6')*H*] and 7.97 [d, J=1.5 Hz, 1H, C(4')*H*]; ^{13}C NMR (100 MHz, CD_3OD) δ 3.5 [$NCH_2CH(CH_2CH_2)$], 6.4 [$NCH_2CH(CH_2CH_2)$], 7.0 [$NCH_2CH(CH_2CH_2)$], 15.0 (CH_3), 25.1 [*C*(10)], 29.8 [*C*(15)], 30.3 [*C*(8)], 36.3 ($CONHCH_2$), 47.6 [*C*(16)], 48.1 [quaternary *C*(13)], 58.9 [*C*(18)], 63.7 [*C*(9)], 73.6 [quaternary *C*(14)], 84.7 [*C*(5)], 110.7 [*C*(7')], 112.2 (quaternary Ar), 119.4 [*C*(2)], 120.0 (Ar), 120.5 (Ar), 122.5 (Ar), 122.6 (quaternary Ar), 125.4 (quaternary Ar), 127.4 (quaternary Ar), 130.0 (quaternary Ar), 132.1 (quaternary Ar), 140.6 (quaternary Ar), 141.9 (quaternary Ar), 144.6 (quaternary Ar) and 171.4 (*C*=O); EI-MS *m/z* 485 [$(M)^+$, 100%], 444 (20) and 430 (15).

2(4-Hydrazinophenyl)ethanol

A solution of 4-aminophenethyl alcohol (1.23 g, 8.98 mmol) in c.HCl (22.5 ml) and H_2O (32 ml) was cooled in an ice-bath. Sodium nitrite (0.71 g, 10.29 mmol) in H_2O (10 ml) was added dropwise with stirring. After stirring at 0 °C for 30 min, the diazonium salt was added to $SnCl_2.H_2O$ (7.91 g) in H_2O (75 ml) at 0 °C and left to stand at 0 °C for 1 h. After basification with NH_4OH, the mixture was centrifuged and the supernatant liquid concentrated *in vacuo*. [CH_3Cl/^{i}PrOH (7:3)] (50 ml) was added and the inorganic salts which precipitated out, were removed by filtration. The solution was partially concentrated and cooled, allowing

crystallisation of the desired product, 4-hydrazinophenethyl alcohol (0.76 g, 4.98 mmol, 55%); mp. 137-138 °C; R_f = 0.47 [$CH_3OH/CH_2Cl_2/NH_4OH$ (10:89:1)]; IR ν_{max}/cm ($CHCl_3$) 3660-2500 (br, OH and NH_2NH); ^1H NMR (270 MHz, $CDCl_3$) δ 2.68 (t, J=7.0 Hz, 2H, ArCH_2), 3.21 (br s, 1H, N*H*), 3.62 (t, J=7.0 Hz, 2H, CH_2OH), 6.85 [d, J=8.4 Hz, 2H, C(3)*H* and C(5)*H*] and 7.12 [d, J=8.4 Hz, 2H, C(2)*H* and C(6)*H*]; ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 39.7 (ArCH_2), 64.5 (CH_2OH), 116.9 [*C*(3 and 5)], 131.3 [C(2 and 6)], 135.7 [*C*(1)] and 144.7 [C(4)]; EI-MS *m/z* 152 [$(M)^+$, 25%], 121 (70) and 106 (80).

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-hydroxyethyl-3,14-dihydroxyindolo-[2',3':6,7]morphinan (101)

4-Hydrazinophenethyl alcohol (0.75 g, 4.93 mmol) and naltrexone (1.45 g, 4.25 mmol) were refluxed under N_2 for 3 h in EtOH/2N HCl (5:1). The solution was concentrated, dissolved in H_2O, basified with NH_4OH and extracted with [$CH_3Cl/^iPrOH$ (7:3)]. The residue was converted to the hydrochloride salt and purified by recrystallisation ($MeOH/CH_2Cl_2$) to give 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-hydroxyethyl-3,14-dihydroxyindolo[2',3':6,7]-morphinan (**101**) (0.16 g, 0.34 mmol, 8%) mp. 186-192 °C; R_f = 0.24 [$CH_3OH/CH_2Cl_2/NH_4OH$ (10:89:1)]; HPLC (C_{18} column) 2.467 [MeOH/0.3% NH_4CO_3 (80:20)]; IR ν_{max}/cm ($CHCl_3$) 3660-2560 (br, NH and OH); ^1H NMR (400 MHz, CD_3OD) δ 0.14-0.24 [m, 2H, $NCH_2CH(CHHCHH)$], 0.50-0.62 [m, 2H, $NCH_2CH(CHHCHH)$], 0.87-0.98 [m, 1H, $NCH_2CH(CH_2CH_2)$], 5.57 [s, 1H, C(5)*H*], 6.52 [d, J=8.2 Hz, 2H, C(1)*H*], 6.55 [d, J=8.2 Hz, 2H, C(2)*H*], 6.96 [d, J=8.2 Hz, 1H, C(6')*H*], 7.21 [s, 1H, *C*(4')] and 7.22 [d, J=8.2 Hz, 1H, C(7')*H*]; ^{13}C NMR (67.8 MHz, CD_3OD) δ 4.2 [$NCH_2CH(CH_2CH_2)$], 4.8 [$NCH_2CH(CH_2CH_2)$], 10.2 [$NCH_2CH(CH_2CH_2)$], 24.2 [*C*(10)], 29.9 [*C*(15)], 32.6 [*C*(8)], 40.5 (CH_2CH_2OH), 45.0 [*C*(16)], 49.1 [quaternary *C*(13)], 60.4 [*C*(18)], 63.6 [*C*(9)], 65.0 (CH_2OH), 74.4 [quaternary *C*(14)], 86.1 [*C*(5)], 110.8 (Ar), 111.9 (Ar), 118.2 (Ar), 119.3 (Ar), 119.6 (Ar), 124.5 (Ar), 125.8 (Ar), 128.1 (Ar), 130.0 (Ar), 130.9 (Ar), 132.0 (Ar), 137.5 (Ar), 140.8 (Ar) and 144.5 (Ar); EI-MS *m/z* 458 [$(M)^+$, 100%] and 106 (90).

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-guanidinylethyl-3,14-dihydroxyindolo-[2',3':6,7]morphinan (104)

Thiourea (1.60 g, 21.1 mmol) was added to NaH (1.75 g, 60% in oil, 43.8 mmol) (prewashed with THF) in THF (80 ml) at 0 °C and the mixture stirred for 10 min. $(BOC)_2O$ (9.40 g, 43.1 mmol) in THF (10 ml) was then added over 30 min. The mixture was allowed to warm to RT and stirred for a further 3 h. The reaction mixture was quenched by stirring with 10% NaOH for 20 min. The organic layer was then separated and the aqueous layer further extracted with EtOAc. The combined organic layers were concentrated and subsequently treated with hexane, which caused the crude product to precipitate out. The crystals were further purified by column chromotography [$CH_3OH/CH_2Cl_2/NH_4OH$ (1:98:1)], to give 1,3-bis-BOC-thiourea (2.27 g, 8.2 mmol, 39%)[75] and 1-mono-BOC-thiourea (1.35 g, 7.7 mmol, 36%). 1,3-Bis-BOC-thiourea ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.52 [s, 18H, 2 x C(C*H*$_3$)]; ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.1 [C(*C*H$_3$)$_3$], 84.1 [*C*(CH$_3$)$_3$], 150.1 (2 x *C*=O) and 177.4 (*C*=S); EI-MS *m/z* 276 [(M)$^+$, 1%] and 57 (100). 1-Mono-BOC-thiourea ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.49 [s, 9H, C(C*H*$_3$)], 6.94 (br s, 1H, N*H*), 8.06 (br s, 1H, N*H*) and 9.21 (br s, 1H, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.7 [C(*C*H$_3$)$_3$], 83.8 [*C*(CH$_3$)$_3$], 151.3 (*C*=O) and 181.9 (*C*=S); EI-MS *m/z* 176 [(M)$^+$, 30%] and 57 (100).

1,3-Bis-BOC-thiourea (0.08 g, 0.30 mmol), 5'-aminoethyl-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-dihydroxyindolo[2',3':6,7]morphinan (**76**) (0.126 g, 0.28 mmol), $HgCl_2$ (0.084 g, 0.30 mmol) and triethylamine (0.028 g, 0.039 ml, 0.28 mmol) were reacted according to general procedure F and stirred at RT for 12 h, to give 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-bis-BOC-guanidinylethyl-3,14-dihydroxyindolo[2',3':6,7]morphinan (**119**) (0.13 g, 0.19 mmol, 67%); Rf = 0.58 [$CH_3OH/CH_2Cl_2/NH_4OH$ (89:10:1)]; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.04-0.17 [m, 2H, NCH$_2$CH(C*H*HC*H*H)], 0.42-0.57 [m, 2H, NCH$_2$CH(CH*H*CH*H*)], 0.75-0.92 [m, 1H, NCH$_2$C*H*(CH$_2$CH$_2$)], 1.36 [s, 9H, C(C*H*$_3$)], 1.43 [s, 9H, C(C*H*$_3$)], 5.60 [s, 1H, C(5)*H*], 6.37 [d, J=8.1 Hz, 1H, C(1)*H*], 6.48 [d, J=8.1 Hz, 1H, C(2)*H*], 6.84 [d, J=8.4 Hz, 1H, C(7')*H*], 7. 06 [d, J=8.4 Hz, 1H, C(6')*H*], 7.15 [s, 1H, C(4')*H*], 7.88 (br s, 1H, N*H*), 8.33 (br s, 1H, N*H*) and 8.74 (br s, 1H, N*H*); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 3.6 [NCH$_2$CH(*C*H$_2$CH$_2$)], 3.9 [NCH$_2$CH(CH$_2$*C*H$_2$)], 9.3 [NCH$_2$*C*H(CH$_2$CH$_2$)], 22.9 [*C*(10)], 27.8 [C(*C*H$_3$)$_3$], 27.9 [C(*C*H$_3$)$_3$], 28.1 (*C*H$_2$CH$_2$NH$_2$), 28.7

(CH_2NH_2), 31.3 [*C*(15)], 36.4 [*C*(8)], 42.8 [*C*(16)], 47.9 [quaternary *C*(13)], 59.3 [*C*(18)], 62.2 [*C*(9)], 72.6 [quaternary *C*(14)], 79.0 [$C(CH_3)_3$], 82.7 [$C(CH_3)_3$], 85.2 [*C*(5)], 110.9 (Ar), 111.3 (Ar), 117.2 (Ar), 118.4 (Ar), 118.6 (Ar), 123.4 (Ar), 124.7 (quaternary Ar), 126.8 (Ar), 128.8 (quaternary Ar), 129.4 (quaternary Ar), 130.6 (quaternary Ar), 136.1 (quaternary Ar), 139.2 (quaternary Ar), 142.9 (quaternary Ar), 152.9 (*C*=O), 155.9 (*C*=O) and 163.4 (*C*=N).

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-bis-Boc-guanidinylethyl-3,14-dihydroxy-indolo[2',3':6,7]morphinan (**119**) (0.13 g, 0.19 mmol) was treated as described in general procedure G, to give 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-guanidinylethyl-3,14-dihydroxyindolo[2',3':6,7]morphinan (**104**) as the bistrifluoroacetic acid salt (0.09 g, 0.19 mmol, 95%); mp. 203-206 °C; Rf = 0.09 [$CH_3OH/CH_2Cl_2/NH_4OH$ (10:89:1)]; IR ν_{max}/cm (KBr) 3650-2500 (br, bonded OH and NH) and 1675 (br, C=N, NH and NH_2); ^{1}H NMR (400 MHz, CD_3OD) δ 0.46-0.57 [m, 2H, $NCH_2CH(CHHCHH)$], 0.71-0.90 [m, 2H, $NCH_2CH(CHHCHH)$], 1.07-1.14 [m, 1H, $NCH_2CH(CH_2CH_2)$], 5.68 [s, 1H, C(5)*H*], 6.65-6.70 [m, 2H, C(1)*H* and C(2)*H*], 7.01-7.06 (m, 1H, Ar*H*) and 7.25-7.33 [m, 2H, Ar*H*]; ^{13}C NMR (100 MHz, CD_3OD) δ 3.5 [$NCH_2CH(CH_2CH_2)$], 6.3 [$NCH_2CH(CH_2CH_2)$], 6.9 [$NCH_2CH(CH_2CH_2)$], 25.0 [*C*(10)], 29.8 ($CH_2CH_2NH_2$), 30.3 (CH_2NH_2), 36.1 [*C*(15)], 44.3 [*C*(8)], 47.5 [*C*(16)], 48.0 [quaternary *C*(13)], 58.9 [*C*(18)], 63.7 [*C*(9)], 73.6 [quaternary *C*(14)], 85.1 [*C*(5)], 109.2 (Ar), 112.5 (Ar), 119.2 (Ar), 119.4 (Ar), 120.4 (Ar), 122.4 (Ar), 124.5 (Ar), 128.0 (Ar), 129.4 (Ar), 130.2 (Ar), 130.7 (Ar), 137.7 (Ar), 141.8 (Ar), 144.6 (Ar) and 158.4 (*C*=NH); FAB$^+$MS *m/z* 500 [$(M+1)^+$, 100%]; HRMS (FAB) *m/z* 500.2670 $(M+1)^+$, $C_{29}H_{34}N_5O_3$ requires 500.2662; Anal. ($C_{29}H_{33}N_5O_3.2TFA.3H_2O$) requires C 50.70, H 5.29, N 8.96, found C 50.60, H 4.98, N 8.63.

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-nitro-3,14-dihydroxyindolo[2',3':6,7]-morphinan (121)

Naltrexone (4.98 g, 14.60 mmol) and 4-nitrophenylhydrazine (2.68 g, 17.52 mmol) were refluxed under N_2, in [EtOH/cHCl (50:50)] (150 ml) for 18 h. After this the solution was concentrated, dissolved in H_2O, basified and extracted with CH_2Cl_2. Further purification was achieved by column chromatography (CH_2Cl_2), to give 17-cyclopropylmethyl-6,7-didehydro-

4,5α-epoxy-5'-nitro-3,14-dihydroxyindolo[2',3':6,7]morphinan (**121**) (2.00 g, 4.35 mmol, 30%); R_f = 0.54 [$CH_3OH/CH_2Cl_2/NH_4OH$ (10:89:1)]; ^1H NMR (400 MHz, $CDCl_3$) δ 0.12-0.25 [m, 2H, $NCH_2CH(CHHCHH)$], 0.48-0.63 [m, 2H, $NCH_2CH(CHHCHH)$], 0.86-0.99 [m, 1H, $NCH_2CH(CH_2CH_2)$], 5.52 [s, 1H, C(5)*H*], 6.45-6.53 [m, 2H, C(1)*H* and C(2)*H*], 7.09-7.16 [m, 1H, Ar*H*] and 7.21-7.30 [m, 2H, 2x Ar*H*]; ^{13}C NMR (100 MHz, $CDCl_3$) δ 4.1 [$NCH_2CH(CH_2CH_2)$] and [$NCH_2CH(CH_2CH_2)$], 9.4 [$NCH_2CH(CH_2CH_2)$], 23.2 [*C*(10)], 28.0 [*C*(15)], 31.4 [*C*(8)], 43.5 [*C*(16)], 48.0 [quaternary *C*(13)], 59.4 [*C*(18)], 62.1 [*C*(9)], 72.8 [quaternary *C*(14)], 84.4 [*C*(5)], 110.0 (Ar), 113.5 (Ar), 114.9 (Ar), 117.0 (Ar), 117.7 (Ar), 119.6 (Ar), 124.7 (Ar), 125.6 (Ar), 130.5 (Ar), 131.6 (Ar), 138.6 (Ar), 139.6 (Ar), 139.7 (Ar) and 142.6 (Ar); FAB-MS *m/z* 460 [$(M)^+$, 100%]; HRMS (FAB) *m/z* 460.1859 $(M)^+$, $C_{26}H_{26}N_3O_5$ requires 460.1872.

5'-Amino-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-dihydroxyindolo[2',3':6,7]-morphinan (120)

To 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-nitro-3,14-dihydroxyindolo[2',3':6,7]-morphinan (**121**) (1.97 g, 4.29 mmol), in [CH_3OH/cyclohexene (50:50)] (140 ml), was added a catalytic amount of Raney nickel. The mixture was stirred at *ca.* 50 °C for 7 h. The mixture was then filtered through a celite pad, concentrated and purified by column chromatography [$CH_3OH/CH_2Cl_2/NH_4OH$ (10:89:1)], yielding 5'-amino-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-dihydroxyindolo[2',3':6,7]morphinan (**120**) (0.81 g, 1.90 mmol, 44%); R_f = 0.41 [$CH_3OH/CH_2Cl_2/NH_4OH$ (10:89:1)]; ^1H NMR (400 MHz, CD_3OD) δ 0.25-0.31 [m, 2H, $NCH_2CH(CHHCHH)$], 0.63-0.70 [m, 2H, $NCH_2CH(CHHCHH)$], 0.87-0.96 [m, 1H, $NCH_2CH(CH_2CH_2)$], 5.70 [s, 1H, C(5)*H*], 6.61 [d, J=8.2 Hz, 1H, C(1)*H*], 6.69 [d, J=8.2 Hz, 1H, C(2)*H*], 7.45 [d, J=9.0 Hz, 1H, C(7')*H*], 8. 08 [dd, J_1=9.0 Hz, J_2=2.1 Hz, 1H, C(6')*H*] and 8.45 [d, J=2.1 Hz, 1H, C(4')*H*]; ^{13}C NMR (100 MHz, CD_3OD) δ 3.3 [$NCH_2CH(CH_2CH_2)$], 3.8 [$NCH_2CH(CH_2CH_2)$], 8.7 [$NCH_2CH(CH_2CH_2)$], 22.9 [*C*(10)], 28.4 [*C*(15)], 30.8 [*C*(8)], 43.6 [*C*(16)], 47.4 [quaternary *C*(13)], 58.8 [*C*(18)], 61.9 [*C*(9)], 72.5 [quaternary *C*(14)], 84.5 [*C*(5)], 104.3 (Ar), 108.8 (Ar), 111.3 (Ar), 113.5 (Ar), 116.6 (Ar), 118.2 (Ar), 123.7 (Ar), 126.8 (Ar), 129.4 (Ar), 130.1 (Ar), 132.2 (Ar), 137.1 (Ar), 139.1 (Ar) and 142.7 (Ar); FAB-MS *m/z* 430 [$(M)^+$, 60%]; HRMS (FAB) *m/z* 430.2119 $(M)^+$, $C_{26}H_{28}N_3O_3$ requires 430.2131.

1,3-Bis-*tert*-butoxycarbonyl-1-benzyl-2-methyl-2-thiopseudourea

1,3-Bis-BOC-2-methyl-2-thiopseudourea (2g, 6.90 mmol), sodium hydride (60% in oil, 0.334 g, 8.36 mmol) and benzyl bromide (1.30 g, 0.90 ml, 7.60 mmol) were reacted according to general procedure E to give 1,3-bis-BOC-1-benzyl-2-methyl-2-thiopseudourea (1.72 g, 4.53 mmol, 66%); Rf = 0.44 [hexane/EtOAc (9:2)]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.36 [s, 9H, C(C*H*$_3$)$_3$], 1.49 [s, 9H, C(C*H*$_3$)$_3$], 2.24 (s, 3H, SC*H*$_3$), 4.74 (s, 2H, C*H*$_2$) and 7.21-7.31 (m, 5H, Ar*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.4 (S*C*H$_3$), 27.8 [C(*C*H$_3$)$_3$], 27.9 [C(*C*H$_3$)$_3$], 52.2 (*C*H$_2$), 81.3 [*C*(CH$_3$)$_3$], 82.3 [*C*(CH$_3$)$_3$], 127.1 (*p*-Ar), 127.3 [*C*(2) and *C*(6) Ar], 128.0 [*C*(3) and *C*(5) Ar], 136.9 (quaternary Ar), 151.5 (*C*=O), 157.4 (*C*=O) and 162.6 (*C*=N); FAB$^-$MS *m/z* 381 [(M+1)$^+$, 75%] and 225 (100); HRMS (FAB) *m/z* 381.1852 (M+1)$^+$, $C_{19}H_{29}N_2O_4S$ requires 381.1848.

1,3-Bis- *tert*-butoxycarbonyl -1-(4'-chlorobenzyl)-2-methyl-2-thiopseudourea[148]

1,3-Bis-BOC-2-methyl-2-thiopseudourea (2g, 6.90 mmol), sodium hydride (60% in oil, 0.334 g, 8.36 mmol) and 4-chlorobenzylbromide (1.56 g, 7 60 mmol) were reacted according to general procedure E, yielding 1,3-bis-BOC-1-(4'-chlorobenzyl)-2-methyl-2-thiopseudourea (2.21 g, 5.33 mmol, 77%) Rf = 0.42 [hexane/EtOAc (9:2)]; ^{1}H NMR (400 MHz, $CDCl_3$) (data in agreement with reference) δ 1.36 [s, 9H, C(C*H*$_3$)$_3$], 1.47 [s, 9H, C(C*H*$_3$)$_3$], 2.24 (s, 3H, SC*H*$_3$), 4.68 (s, 2H, C*H*$_2$) and 7.24 (s, 4H, Ar*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.0 (S*C*H$_3$), 28.3 [C(*C*H$_3$)$_3$], 28.4 [C(*C*H$_3$)$_3$], 52.0 (*C*H$_2$), 82.0 [*C*(CH$_3$)$_3$], 83.1 [*C*(CH$_3$)$_3$], 128.7 [*C*(3) and *C*(5) Ar], 129.4 [*C*(2) and *C*(6) Ar], 133.4 (quaternary Ar), 136.0 (quaternary Ar), 151.9 (*C*=O), 157.9 (*C*=O) and 162.7 (*C*=N); FAB$^+$MS *m/z* 415 [(M+1)$^+$, 30%] and 259 (65); HRMS (FAB) *m/z* 415.1439 (M+1)$^+$, $C_{19}H_{28}N_2O_4SCl$ requires 415.1458.

1,3-Bis- *tert*-butoxycarbonyl -1-(4'-nitrobenzyl)-2-methyl-2-thiopseudourea

1,3-Bis-BOC-2-methyl-2-thiopseudourea (2g, 6.90 mmol), sodium hydride (60% in oil, 0.334 g, 8.36 mmol) and 4-nitrobenzylbromide (1.64 g, 7.60 mmol) were reacted according to general procedure E, giving 1,3-bis-BOC-1-(4'-nitrobenzyl)-2-methyl-2-thiopseudourea (2.20 g, 5.18

mmol, 75%) Rf = 0.36 [hexane/EtOAc (9:2)]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.38 [s, 9H, C(C*H*$_3$)$_3$], 1.50 [s, 9H, C(C*H*$_3$)$_3$], 2.33 (s, 3H, SC*H*$_3$), 4.84 (s, 2H, C*H*$_2$), 7.50 [d, J 8.6, 2H, C(2)*H* and C(6)*H*] and 8.17 [d, J 8.6, 2H, C(3)*H* and C(5)*H*]; ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.7 (S*C*H$_3$), 28.0 [C(*C*H$_3$)$_3$], 28.1 [C(*C*H$_3$)$_3$], 51.7 (*C*H$_2$), 82.0 [*C*(CH$_3$)$_3$], 83.4 [*C*(CH$_3$)$_3$], 123.5 [*C*(3) and *C*(5) Ar], 128.2 [*C*(2) and *C*(6) Ar], 144.7 [*C*(1) quaternary Ar], 147.1 [*C*(4) quaternary Ar], 151.5 (*C*=O), 157.4 (*C*=O) and 161.9 (*C*=N); FAB$^-$MS *m/z* 426 [(M+1)$^+$, 55%] and 270 (100); HRMS (FAB) *m/z* 426.1699 (M+1)$^+$, $C_{19}H_{28}N_3O_6S$ requires 426.1699.

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N'-benzyl)guanidinylethyl-3,14-dihydroxyindolo[2',3':6,7]morphinan (106)

1,3-Bis-BOC-1-benzyl-2-methyl-2-thiopseudourea (0.208 g, 0.53 mmol), 5'-aminoethyl-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-dihydroxyindolo[2',3':6,7]morphinan (**76**) (0.123 g, 0.27 mmol), $HgCl_2$ (0.081 g, 0.30 mmol) and triethylamine (0.055 g, 0.075 ml, 0.54 mmol) were reacted according to general procedure F and stirred at 50 °C for 12 h, to give 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-bis-BOC-(N'-benzyl)guanidinylethyl-3,14-dihydroxyindolo[2',3':6,7]morphinan (0.050 g, 0.06 mmol, 24%); Rf = 0.63 [$CH_3OH/CH_2Cl_2/NH_4OH$ (89:10:1)]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.26-0.34 [m, 2H, NCH$_2$CH(C*H*HC*H*H)], 0.64-0.73 [m, 2H, NCH$_2$CH(CH*H*CH*H*)], 0.96-1.05 [m, 1H, NCH$_2$C*H*(CH$_2$CH$_2$)], 1.29 [s, 9H, C(C*H*$_3$)$_3$], 1.33 [s, 9H, C(C*H*$_3$)$_3$], 5.75 [s, 1H, C(5)*H*], 6.58 [d, J=8.2 Hz, 1H, C(1)*H*], 6.68 [d, J=8.2 Hz, 1H, C(2)*H*], 6.82-6.91 [m, 1H, Ar*H*] and 7.15-7.47 [m, 7H, Ar*H*]; ^{13}C NMR (100 MHz, $CDCl_3$) δ 3.9 [NCH$_2$CH(*C*H$_2$CH$_2$)], 4.2 [NCH$_2$CH(CH$_2$*C*H$_2$)], 9.5 [NCH$_2$*C*H(CH$_2$CH$_2$)], 23.3 [*C*(10)], 28.2 [C(*C*H$_3$)$_3$], 28.3 [C(*C*H$_3$)$_3$], 28.9 (*C*H$_2$CH$_2$NH$_2$), 31.4 (*C*H$_2$NH$_2$), 31.5 [*C*(15)], 36.5 [*C*(8)], 43.8 [*C*(16)], 46.1 (benzylic *C*H$_2$), 48.1 [quaternary *C*(13)], 59.5 [*C*(18)], 62.3 [*C*(9)], 72.6 [quaternary *C*(14)], 79.2 [*C*(CH$_3$)$_3$], 82.4 [*C*(CH$_3$)$_3$], 85.2 [*C*(5)], 111.0 (Ar), 111.3 (Ar), 117.1 (Ar), 118.6 (Ar), 118.7 (Ar), 123.3 (Ar), 124.7 (Ar), 126.8 (Ar), 127.3 (Ar), 128.3 (Ar), 129.3 (Ar), 130.5 (Ar), 136.0 (Ar), 137.5 (Ar), 139.1 (Ar), 142.8 (Ar) and 162.4 (*C*=N); FAB$^+$MS *m/z* 790 [(M+1)$^+$, 70%] and 590 (100).

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-bis-Boc-(N'-benzyl)guanidinylethyl-3,14-dihydroxyindolo[2',3':6,7]morphinan (0.042 g, 0.05 mmol) was treated as described in general procedure G, to give 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N'-benzyl)guanidinyl-ethyl-3,14-dihydroxyindolo[2',3':6,7]morphinan (**106**) as the bistrifluoroacetic acid salt (0.037 g, 0.045 mmol, 85%); mp. 169 °C; Rf = 0.21 [$CH_3OH/CH_2Cl_2/NH_4OH$ (89:10:1)]; IR ν_{max}/cm (KBr) 3680-2460 (br, bonded OH and NH) and 1660 (br, C=N, NH and NH_2); ^1H NMR (400 MHz, CD_3OD) δ 0.46-0.61 [m, 2H, $NCH_2CH(CHHCHH)$], 0.70-0.92 [m, 2H, $NCH_2CH(CHHCHH)$], 1.07-1.21 [m, 1H, $NCH_2CH(CH_2CH_2)$], 5.70 [s, 1H, C(5)*H*], 6.65 [s, 2H, C(1)*H* and C(2)*H*] and 6.98-7.32 [m, 8H, Ar*H*]; ^{13}C NMR (100 MHz, CD_3OD) δ 3.4 [$NCH_2CH(CH_2CH_2)$], 6.2 [$NCH_2CH(CH_2CH_2)$], 6.9 [$NCH_2CH(CH_2CH_2)$], 25.0 [*C*(10)], 29.8 ($CH_2CH_2NH_2$), 30.4 (CH_2NH_2), 36.2 [*C*(15)], 44.4 [*C*(8)], 45.8 [*C*(16)], 47.6 [quaternary *C*(13)], 48.1 (benzylic CH_2), 58.9 [*C*(18)], 63.7 [*C*(9)], 73.7 [quaternary *C*(14)], 85.2 [*C*(5)], 109.3 (Ar), 112.7 (Ar), 119.4 (Ar), 119.5 (Ar), 120.4 (Ar), 122.5 (Ar), 124.6 (Ar), 127.9 (2 x Ar), 128.2 (Ar), 128.8 (Ar), 129.5 (Ar), 129.7 (2 x Ar), 130.3 (Ar), 130.8 (Ar), 137.4 (Ar), 137.9 (Ar), 142.0 (Ar), 144.8 (Ar) and 157.4 (*C*=NH); FAB$^+$MS *m/z* 590 [$(M+1)^+$, 100%]; HRMS (FAB) *m/z* 590.3163 $(M+1)^+$, $C_{36}H_{40}N_5O_3$ requires 590.3131; Anal. ($C_{36}H_{39}N_5O_3.2TFA.3.75H_2O$) requires C 54.27, H 5.52, N 7.91, found C 53.90, H 4.93, N 7.65.

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N'-4-chlorobenzyl)guanidinylethyl-3,14-dihydroxyindolo[2',3':6,7]morphinan (110)

1,3-Bis-BOC-1-(4'-chlorobenzyl)-2-methyl-2-thiopseudourea (0.318 g, 0.77 mmol), 5'-aminoethyl-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-dihydroxyindolo[2',3':6,7]-morphinan (**76**) (0.177 g, 0.39 mmol), $HgCl_2$ (0.106 g, 0.39 mmol) and triethylamine (0.078 g, 0.110 ml, 0.77 mmol) were reacted according to general procedure F and stirred at 50 °C for 12 h, to give 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-bis-BOC-(N'-4-chlorobenzyl)-guanidinylethyl-3,14-dihydroxyindolo[2',3':6,7]morphinan (0.088 g, 0.11 mmol, 28%); Rf = 0.36 [$CH_3OH/CH_2Cl_2/NH_4OH$ (89:10:1)]; ^1H NMR (400 MHz, $CDCl_3$) δ 0.32-0.39 [m, 2H, $NCH_2CH(CHHCHH)$], 0.72-0.80 [m, 2H, $NCH_2CH(CHHCHH)$], 1.04-1.12 [m, 1H, $NCH_2CH(CH_2CH_2)$], 1.50 [s, 9H, $C(CH_3)_3$], 1.63 [s, 9H, $C(CH_3)_3$], 5.84 [s, 1H, C(5)*H*], 6.64 [d,

J=8.2 Hz, 1H, C(1)*H*], 6.73 [d, J=8.2 Hz, 1H, C(2)*H*], 6.40-6.52 [m, 1H, Ar*H*] and 7.24-7.49 [m, 6H, Ar*H*]; ^{13}C NMR (100 MHz, $CDCl_3$) δ 4.2 [$NCH_2CH(CH_2CH_2)$], 4.6 [$NCH_2CH(CH_2CH_2)$], 9.8 [$NCH_2CH(CH_2CH_2)$], 23.6 [*C*(10)], 28.5 [$C(CH_3)_3$], 28.6 [$C(CH_3)_3$], 29.2 ($CH_2CH_2NH_2$), 31.8 (CH_2NH_2), 31.9 [*C*(15)], 36.9 [*C*(8)], 44.1 [*C*(16)], 46.5 (benzylic CH_2), 48.4 [quaternary *C*(13)], 59.8 [*C*(18)], 62.6 [*C*(9)], 73.0 [quaternary *C*(14)], 79.7 [$C(CH_3)_3$], 83.2 [$C(CH_3)_3$], 85.6 [*C*(5)], 111.3 (Ar), 111.8 (Ar), 117.5 (Ar), 118.9 (Ar), 119.1 (Ar), 123.5 (Ar), 125.1 (Ar), 127.2 (Ar), 128.4 (Ar), 128.8 (Ar), 129.7 (Ar), 130.0 (Ar), 130.9 (Ar), 133.4 (Ar), 136.4 (Ar), 139.4 (Ar), 143.1 (Ar) and 162.8 (*C*=NH); FAB$^+$MS *m/z* 824 [$(M+1)^+$, 95%] and 624 (100).

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-bis-BOC-(N'-4-chlorobenzyl)guanidinylethyl-3,14-dihydroxyindolo[2',3':6,7]morphinan (0.088 g, 0.11 mmol) was treated as described in general procedure G, to give 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N'-4-chlorobenzyl)guanidinyl-ethyl-3,14-dihydroxyindolo[2',3':6,7]morphinan (**110**) as the bistrifluoroacetic acid salt (0.089 g, 0.10 mmol, 98%); mp. 204-205 °C; Rf = 0.26 [$CH_3OH/CH_2Cl_2/NH_4OH$ (89:10:1)]; IR ν_{max}/cm (KBr) 3680-2500 (br, bonded OH and NH) and 1660 (br, C=N, NH and NH_2); ^{1}H NMR (400 MHz, CD_3OD) δ 0.47-0.59 [m, 2H, $NCH_2CH(CHHCHH)$], 0.71-0.93 [m, 2H, $NCH_2CH(CHHCHH)$], 1.05-1.21 [m, 1H, $NCH_2CH(CH_2CH_2)$], 5.70 [s, 1H, C(5)*H*], 6.67 [s, 2H, C(1)*H* and C(2)*H*], 7.00 [d, J=8.2 Hz, 1H, C(6')*H*], 7.12 [d, J=8.2 Hz, 1H, C(7')*H*], 7.13 (s, 1H, C(4')*H*] and 7.24-7.36 [m, 4H, Ar*H*]; ^{13}C NMR (100 MHz, CD_3OD) δ 3.5 [$NCH_2CH(CH_2CH_2)$], 6.3 [$NCH_2CH(CH_2CH_2)$], 6.9 [$NCH_2CH(CH_2CH_2)$], 25.0 [*C*(10)], 29.8 ($CH_2CH_2NH_2$), 30.3 (CH_2NH_2), 36.2 [*C*(15)], 44.5 [*C*(8)], 45.1 [*C*(16)], 47.5 [quaternary *C*(13)], 48.0 (benzylic CH_2), 58.8 [*C*(18)], 63.6 [*C*(9)], 73.6 [quaternary *C*(14)], 85.1 [*C*(5)], 109.2 (Ar), 112.6 (Ar), 119.2 (Ar), 119.5 (Ar), 120.4 (Ar), 122.4 (Ar), 124.5 (Ar), 128.0 (Ar), 129.4 (Ar), 129.5 (2 x Ar), 129.6 (2 x Ar), 130.2 (Ar), 130.7 (Ar), 134.4 (Ar), 136.3 (Ar), 137.7 (Ar), 141.8 (Ar), 144.6 (Ar) and 157.3 (*C*=NH); FAB$^+$MS *m/z* 624 [$(M+1)^+$, 100%]; HRMS (FAB) *m/z* 624.2721 $(M+1)^+$, $C_{36}H_{39}N_5O_3Cl$ requires 624.2741; Anal. ($C_{36}H_{38}N_5O_3Cl.2TFA.3H_2O$) requires C 53.01, H 5.12, N 7.73, found C 53.20, H 4.89, N 7.77.

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N'-4-nitrobenzyl)guanidinylethyl-3,14-dihydroxyindolo[2',3':6,7]morphinan (111)

1,3-Bis-BOC-1-(4'-nitrobenzyl)-2-methyl-2-thiopseudourea (0.381 g, 0.90 mmol), 5'-aminoethyl-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-dihydroxyindolo[2',3':6,7]morphinan (**76**) (0.206 g, 0.45 mmol), $HgCl_2$ (0.122 g, 0.45 mmol) and triethylamine (0.091 g, 0.125 ml, 0.90 mmol) were reacted according to general procedure F and stirred at 50 °C for 12 h, to give 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-bis-BOC-(N'-4-nitrobenzyl)guanidinylethyl-3,14-dihydroxyindolo[2',3':6,7]morphinan (0.167 g, 0.20 mmol, 44%); Rf = 0.57 [$CH_3OH/CH_2Cl_2/NH_4OH$ (89:10:1)]; ^1H NMR (400 MHz, $CDCl_3$) δ 0.16-0.23 [m, 2H, $NCH_2CH(CHHCHH)$], 0.54-0.63 [m, 2H, $NCH_2CH(CHHCHH)$], 0.87-0.96 [m, 1H, $NCH_2CH(CH_2CH_2)$], 1.33 [s, 9H, $C(CH_3)_3$], 1.49 [s, 9H, $C(CH_3)_3$], 5.70 [s, 1H, C(5)*H*], 6.50 [d, J=8.0 Hz, 1H, C(1)*H*], 6.58 [d, J=8.0 Hz, 1H, C(2)*H*], 6.75-6.87 [m, 1H, C(4')*H*], 7.07-7.28 [m, 2H, C(6')*H* and C(7')*H*] and 7.95-8.07 [m, 4H, Ar*H*]; ^{13}C NMR (100 MHz, $CDCl_3$) δ 4.2 [$NCH_2CH(CH_2CH_2)$], 4.6 [$NCH_2CH(CH_2CH_2)$], 9.9 [$NCH_2CH(CH_2CH_2)$], 23.5 [*C*(10)], 28.4 [$C(CH_3)_3$], 28.6 [$C(CH_3)_3$], 29.1 ($CH_2CH_2NH_2$), 31.9 (CH_2NH_2) and [*C*(15)], 36.9 [*C*(8)] and (benzylic CH_2), 44.1 [*C*(16)], 48.4 [quaternary *C*(13)], 59.8 [*C*(18)], 62.5 [*C*(9)], 73.0 [quaternary *C*(14)], 79.8 [$C(CH_3)_3$], 83.6 [$C(CH_3)_3$], 85.5 [*C*(5)], 111.2 (Ar), 111.7 (Ar), 117.4 (Ar), 118.8 (Ar), 119.0 (Ar), 123.4 (Ar), 123.7 (Ar), 125.0 (Ar), 127.1 (Ar), 128.2 (Ar), 128.8 (Ar), 130.0 (Ar), 131.0 (Ar), 136.4 (Ar), 139.5 (Ar), 143.2 (Ar), 147.1 (Ar) and 162.8 (*C*=NH); FAB^+MS *m/z* 835 [$(M+1)^+$, 100%] and 635 (95); HRMS (FAB) *m/z* 835.4022 $(M+1)^+$, $C_{46}H_{54}N_6O_9$ requires 835.4030.

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-bis-BOC-(N'-4-nitrobenzyl)guanidinylethyl-3,14-dihydroxyindolo[2',3':6,7]morphinan (0.075 g, 0.09 mmol) was treated as described in general procedure G, to give 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N'-4-nitrobenzyl)guanidinyl ethyl-3,14-dihydroxyindolo[2',3':6,7]morphinan (**111**) as the bistrifluoroacetic acid salt (0.078 g, 0.09 mmol, 99%); mp. 187-190 °C; Rf = 0.21 [$CH_3OH/CH_2Cl_2/NH_4OH$ (89:10:1)]; IR ν_{max}/cm (KBr) 3660-2540 (br, bonded OH and NH) and 1665 (br, C=N, NH and NH_2); ^1H NMR (400 MHz, CD_3OD) δ 0.46-0.62 [m, 2H,

$NCH_2CH(CHHCHH)$], 0.72-0.93 [m, 2H, $NCH_2CH(CHHCHH)$], 1.06-1.21 [m, 1H, $NCH_2CH(CH_2CH_2)$], 5.76 [s, 1H, C(5)*H*], 6.61-6.74 [m, 2H, C(1)*H* and C(2)*H*], 7.03 [d, J=8.2 Hz, 1H, C(6')*H*], 7.22 [d, J=8.2 Hz, 1H, C(7')*H*], 7.25-7.36 [m, 3H, C(4')*H* and Ar*H*] and 8.00-8.13 (m, 2H, Ar*H*); ^{13}C NMR (100 MHz, CD_3OD) δ 3.5 [$NCH_2CH(CH_2CH_2)$], 6.3 [$NCH_2CH(CH_2CH_2)$], 6.9 [$NCH_2CH(CH_2CH_2)$], 25.1 [*C*(10)], 29.8 ($CH_2CH_2NH_2$), 30.3 (CH_2NH_2), 36.3 [*C*(15)], 44.5 [*C*(8)], 45.0 [*C*(16)], 47.6 [quaternary *C*(13)], 48.0 (benzylic CH_2), 58.9 [*C*(18)], 63.7 [*C*(9)], 73.7 [quaternary *C*(14)], 85.1 [*C*(5)], 109.2 (Ar), 112.6 (Ar), 119.2 (Ar), 119.6 (Ar), 120.3 (Ar), 122.4 (Ar), 124.5 (Ar), 124.6 (Ar), 128.0 (Ar), 128.5 (2 x Ar), 129.5 (Ar), 130.2 (Ar), 130.8 (Ar), 137.8 (Ar), 141.8 (Ar), 144.7 (Ar), 145.2 (Ar) and 157.4 (*C*=NH); FAB^+MS *m/z* 635 [$(M+1)^+$, 100%]; HRMS (FAB) *m/z* 635.2990 $(M+1)^+$, $C_{36}H_{39}N_6O_5$ requires 635.2982; Anal. ($C_{36}H_{38}N_6O_5.2TFA.2.5H_2O$) requires C 52.92, H 5.00, N 9.26, found C 52.70, H 4.76, N 9.34.

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N'-4-aminobenzyl)guanidinylethyl-3,14-dihydroxyindolo[2',3':6,7]morphinan (112)

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-bis-BOC-(N'-4-nitrobenzyl)guanidinylethyl-3,14-dihydroxyindolo[2',3':6,7]morphinan (0.082 g, 0.10 mmol) was dissolved in CH_3OH (30 ml) and cyclohexene (30 ml). To this was added a few drops of triethylamine and a catalytic amount of Raney Nickel catalyst. The biphasic solution was stirred at *ca.* 30 °C for 3 h, filtered through a cellite pad, and concentrated to yield the crude product, which was then purified by column chromatography [$CH_3OH/CH_2Cl_2/NH_4OH$ (89:10:1)] to afford 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-bis-BOC-(N'-4-aminobenzyl)guanidinylethyl-3,14-dihydroxyindolo-[2',3':6,7]morphinan (**127**) (0.055 g, 0.07 mmol, 70%); Rf = 0.42 [$CH_3OH/CH_2Cl_2/NH_4OH$ (89:10:1)]; ^1H NMR (400 MHz, $CDCl_3$) δ 0.13-0.21 [m, 2H, $NCH_2CH(CHHCHH)$], 0.52-0.62 [m, 2H, $NCH_2CH(CHHCHH)$], 0.84-0.93 [m, 1H, $NCH_2CH(CH_2CH_2)$], 1.46 [s, 9H, $C(CH_3)_3$], 1.48 [s, 9H, $C(CH_3)_3$], 5.65 [s, 1H, C(5)*H*], 6.25-6.38 (m, 1H, Ar*H*), 6.50 [d, J=8.2 Hz, 1H, C(1)*H*], 6.58 [d, J=8.2 Hz, 1H, C(2)*H*] and 6.74-7.24 (m, 6H, Ar*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 3.9 [$NCH_2CH(CH_2CH_2)$], 4.2 [$NCH_2CH(CH_2CH_2)$], 9.4 [$NCH_2CH(CH_2CH_2)$], 23.3 [*C*(10)], 28.2 [$C(CH_3)_3$], 28.3 [$C(CH_3)_3$], 28.9 ($CH_2CH_2NH_2$), 29.8 (CH_2NH_2), 31.4 [*C*(15)], 37.2 [*C*(8)], 43.8 [*C*(16)], 46.0 (benzylic CH_2), 48.0 [quaternary *C*(13)], 59.4 [*C*(18)], 62.3 [*C*(9)], 72.8 [quaternary

C(14)], 79.2 [*C*(CH_3)$_3$], 82.2 [*C*(CH_3)$_3$], 85.2 [*C*(5)], 110.9 (Ar), 111.3 (Ar), 115.1 (2 x Ar), 115.3 (Ar), 117.5 (Ar), 118.5 (Ar), 118.9 (Ar), 119.8 (Ar), 123.4 (Ar), 124.5 (Ar), 126.8 (Ar), 128.4 (Ar), 129.3 (2 x Ar), 130.4 (Ar), 136.1 (Ar), 139.2 (Ar), 142.7 (Ar), 145.3 (Ar) and 156.1 (*C*=NH); FAB^+MS *m/z* 805 [$(M+1)^+$, 30%], 700 (75) and 500 (100); HRMS (FAB) *m/z* 805.4273 $(M+1)^+$, $C_{46}H_{57}N_6O_7$ requires 805.4289.

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-bis-BOC-(N'-4-aminobenzyl)guanidinyl-ethyl-3,14-dihydroxyindolo[2',3':6,7]morphinan (**126**) (0.055 g, 0.07 mmol) was treated as described in general procedure G, to give 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N'-4-aminobenzyl)guanidinylethyl-3,14-dihydroxyindolo[2',3':6,7]morphinan (**112**) as the tristrifluoroacetic acid salt (0.051 g, 0.05 mmol, 79%); mp. 184-188 °C; Rf = 0.18 [$CH_3OH/CH_2Cl_2/NH_4OH$ (89:10:1)]; IR ν_{max}/cm (KBr) 3680-2400 (br, bonded OH and NH) and 1670 (br, C=N, NH and NH_2); ^1H NMR (400 MHz, CD_3OD) δ 0.48-0.61 [m, 2H, $NCH_2CH(CHHCHH)$], 0.72-0.93 [m, 2H, $NCH_2CH(CHHCHH)$], 1.09-1.22 [m, 1H, $NCH_2CH(CH_2CH_2)$], 5.73 [s, 1H, C(5)*H*], 6.65 [d, J=8.2 Hz, 1H, C(1)*H*], 6.67 [d, J=8.2 Hz, 1H, C(2)*H*], 6.99-7.06 (m, 1H, Ar*H*) and 7.21-7.35 (m, 6H, Ar*H*); ^{13}C NMR (100 MHz, CD_3OD) δ 3.5 [$NCH_2CH(CH_2CH_2)$], 6.3 [$NCH_2CH(CH_2CH_2)$], 6.9 [$NCH_2CH(CH_2CH_2)$], 25.1 [*C*(10)], 29.9 ($CH_2CH_2NH_2$), 30.4 (CH_2NH_2), 36.3 [*C*(15)], 44.6 [*C*(8)], 45.1 [*C*(16)], 47.6 [quaternary *C*(13)], 48.1 (benzylic CH_2), 58.9 [*C*(18)], 63.7 [*C*(9)], 73.7 [quaternary *C*(14)], 85.2 [*C*(5)], 109.3 (Ar), 112.6 (Ar), 119.2 (Ar), 119.5 (Ar), 120.4 (Ar), 120.9 (Ar), 122.5 (Ar), 123.1 (2Ar), 124.6 (Ar), 128.1 (Ar), 129.0 (Ar), 129.4 (Ar), 129.5 (2Ar), 130.2 (Ar), 130.7 (Ar), 137.8 (Ar), 141.7 (Ar), 144.6 (Ar) and 157.3 (*C*=NH); FAB^+MS *m/z* 605 [$(M+1)^+$, 80%] and 500 (80); HRMS (FAB) *m/z* 605.3232 $(M+1)^+$, $C_{36}H_{41}N_6O_3$ requires 605.3240; Anal. ($C_{36}H_{40}N_6O_3.3TFA.1H_2O$) requires C 52.28, H 4.70, N 8.71, found C 52.50, H 4.90, N 8.47.

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N'-benzyl)guanidinyl-3,14-dihydroxy-indolo[2',3':6,7]morphinan (105)

1,3-Bis-BOC-1-benzyl-2-methyl-2-thiopseudourea (0.180 g, 0.46 mmol), 5'-amino-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-dihydroxy-indolo[2',3':6,7]morphinan (**120**)

(0.099 g, 0.23 mmol), $HgCl_2$ (0.063 g, 0.23 mmol) and triethylamine (0.047 g, 0.065 ml, 0.46 mmol) were reacted according to general procedure F and stirred at 50 °C for 24 h, to give 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-bis-BOC-(N'-benzyl)guanidinyl-3,14-dihydroxy-indolo[2',3':6,7]morphinan (0.053 g, 0.07 mmol, 30%); Rf = 0.50 [$CH_3OH/CH_2Cl_2/NH_4OH$ (89:10:1)]; ^1H NMR (270 MHz, $CDCl_3$) δ 0.08-0.21 [m, 2H, $NCH_2CH(CHHCHH)$], 0.48-0.63 [m, 2H, $NCH_2CH(CHHCHH)$], 0.80-0.92 [m, 1H, $NCH_2CH(CH_2CH_2)$], 1.35 [s, 9H, $C(CH_3)_3$], 1.42 [s, 9H, $C(CH_3)_3$], 5.56 [s, 1H, C(5)*H*], 6.46 [d, J=7.8 Hz, 1H, C(1)*H*], 6.54 [d, J=7.8 Hz, 1H, C(2)*H*], 6.56-6.65 [m, 1H, Ar*H*], 6.70-6.79 [s, 1H, Ar*H*], 6.92-7.02 [m, 1H, Ar*H*] and 7.10-7.49 [m, 5H, Ar*H*]; ^{13}C NMR (100 MHz, $CDCl_3$) δ 3.9 [$NCH_2CH(CH_2CH_2)$], 4.1 [$NCH_2CH(CH_2CH_2)$], 9.5 [$NCH_2CH(CH_2CH_2)$], 23.3 [*C*(10)], 27.7 [$C(CH_3)_3$], 28.0 [*C*(15)], 28.2 [$C(CH_3)_3$], 28.9 [*C*(8)], 43.7 [*C*(16)], 48.0 (CH_2Ph), 51.0 [quaternary *C*(13)], 59.4 [*C*(18)], 62.3 [*C*(9)], 72.3 [quaternary *C*(14)], 81.6 [$C(CH_3)_3$], 82.5 [$C(CH_3)_3$], 84.9 [*C*(5)], 111.3 (Ar), 111.5 (Ar), 112.5 (Ar), 117.2 (Ar), 118.7 (Ar), 124.5 (Ar), 126.6 (Ar), 126.9 (Ar), 127.3 (Ar), 127.9 (Ar), 128.2 (2 x Ar), 128.7 (Ar), 128.9 (2 x Ar), 130.1 (Ar), 130.5 (Ar), 135.1 (Ar), 137.5 (Ar), 139.3 (Ar), 142.7 (C=O), 152.6 (C=O) and 153.4 (*C*=N); FAB^+MS *m/z* 762 [$(M+1)^+$, 100%], 662 (10) and 562 (20); HRMS (FAB) *m/z* 762.3859 $(M+1)^+$, $C_{44}H_{52}N_5O_7$ requires 762.3867.

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-bis-BOC-(N'-benzyl)guanidinyl-3,14-dihydroxyindolo[2',3':6,7]morphinan (0.053 g, 0.07 mmol) was treated as described in general procedure G, to give 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N'-benzyl)guanidinyl-3,14-dihydroxyindolo [2',3':6,7]morphinan (**105**) as the bistrifluoroacetic acid salt (0.046 g, 0.07 mmol, 84%); mp. 180-182 °C; Rf = 0.17 [$CH_3OH/CH_2Cl_2/NH_4OH$ (89:10:1)]; IR ν_{max}/cm (KBr) 3710-2450 (br, bonded OH and NH) and 1678 (br, C=N, NH and NH_2); ^1H NMR (400 MHz, CD_3OD) δ 0.49-0.59 [m, 2H, $NCH_2CH(CHHCHH)$], 0.72-0.92 [m, 2H, $NCH_2CH(CHHCHH)$], 1.09-1.23 [m, 1H, $NCH_2CH(CH_2CH_2)$], 5.72 [s, 1H, C(5)*H*], 6.64 [d, J=8.2 Hz, 1H, C(1)*H*], 6.67 [d, J=8.2 Hz, 1H, C(2)*H*], 6.99-7.03 (m, 1H, Ar*H*) and 7.28-7.46 [m, 7H, Ar*H*]; ^{13}C NMR (100 MHz, CD_3OD) δ 3.5 [$NCH_2CH(CH_2CH_2)$], 6.3 [$NCH_2CH(CH_2CH_2)$], 6.9 [$NCH_2CH(CH_2CH_2)$], 25.1 [*C*(10)], 29.8 [*C*(15)], 30.3 [*C*(8)], 46.0 [*C*(16)], 47.6 [quaternary *C*(13)], 48.1 (CH_2Ph), 58.9 [*C*(18)], 63.6 [*C*(9)], 73.5 [quaternary *C*(14)], 84.8 [*C*(5)], 109.9 (Ar), 113.7 (Ar), 118.1 (Ar), 119.2 (Ar), 120.4 (Ar), 122.2 (Ar), 122.4 (Ar), 126.7 (Ar), 128.0 (2 x Ar), 128.4 (Ar), 128.8 (Ar),

129.7 (2 x Ar), 130.1 (Ar), 132.3 (Ar), 137.5 (Ar), 138.0 (Ar), 141.9 (Ar), 144.6 (Ar) and 157.5 (*C*=NH); FAB$^+$MS *m/z* 562 [(M+1)$^+$, 30%]; HRMS (FAB) *m/z* 562.2815 (M+1)$^+$, $C_{34}H_{36}N_5O_3$ requires 562.2818.

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N'-4-chlorobenzyl)guanidinyl-3,14-dihydroxyindolo[2',3':6,7]morphinan (107)

1,3-Bis-BOC-1-(4'-chlorobenzyl-2-methyl-2-thiopseudourea (0.243 g, 0.59 mmol), 5'-amino-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-dihydroxyindolo[2',3':6,7]morphinan (**120**) (0.126 g, 0.29 mmol), $HgCl_2$ (0.079 g, 0.29 mmol) and triethylamine (0.059 g, 0.082 ml, 0.59 mmol) were reacted according to general procedure F and stirred at 50 °C for 24 h, to give 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-bis-BOC-(N'-4-chlorobenzyl)guanidinyl-3,14-dihydroxyindolo[2',3':6,7]morphinan (0.103 g, 0.13 mmol, 44%); Rf = 0.47 [$CH_3OH/CH_2Cl_2/NH_4OH$ (89:10:1)]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.16-0.27 [m, 2H, $NCH_2CH(CHHCHH)$], 0.56-0.68 [m, 2H, $NCH_2CH(CHHCHH)$], 0.88-1.00 [m, 1H, $NCH_2CH(CH_2CH_2)$], 1.37 [s, 9H, $C(CH_3)_3$], 1.43 [s, 9H, $C(CH_3)_3$], 5.60 [s, 1H, C(5)*H*], 6.55 [d, J=8.2 Hz, 1H, C(1)*H*], 6.59 [d, J=8.2 Hz, 1H, C(2)*H*], 6.70 (s, 1H, C(4')*H*], 6.78 [d, J=8.6 Hz, 1H, C(6')*H*], 7.21 [d, J=8.6 Hz, 1H, C(7')*H*] and 7.25-7.48 [m, 4H, Ar*H*]; ^{13}C NMR (100 MHz, $CDCl_3$) δ 3.4 [$NCH_2CH(CH_2CH_2)$], 3.9 [$NCH_2CH(CH_2CH_2)$], 9.2 [$NCH_2CH(CH_2CH_2)$], 22.9 [*C*(10)], 27.2 [$C(CH_3)_3$], 27.6 [*C*(15)], 27.7 [$C(CH_3)_3$], 28.4 [*C*(8)], 43.5 [*C*(16)], 47.6 (CH_2Ph), 47.9 [quaternary *C*(13)], 59.1 [*C*(18)], 61.8 [*C*(9)], 72.3 [quaternary *C*(14)], 79.7 [$C(CH_3)_3$], 82.6 [$C(CH_3)_3$], 84.3 [*C*(5)], 110.2 (Ar), 111.2 (Ar), 112.0 (Ar), 116.6 (Ar), 118.1 (Ar), 118.4 (Ar), 124.2 (Ar), 126.3 (Ar), 127.8 (Ar), 128.2 (2 x Ar), 129.9 (Ar), 130.1 (2 x Ar), 130.2 (Ar), 130.4 (Ar), 132.8 (Ar), 135.1 (Ar), 135.3 (Ar), 139.0 (Ar), 142.6 (C=O) and 162.7 (*C*=N); FAB$^+$MS *m/z* 796 [(M+1)$^+$, 100%], 696 (20) and 596 (30); HRMS (FAB) *m/z* 796.3463 (M+1)$^+$, $C_{44}H_{51}N_5O_7Cl$ requires 796.3477.

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-bis-BOC-(N'-4-chlorobenzyl)guanidinyl-3,14-dihydroxyindolo[2',3':6,7]morphinan (0.100 g, 0.13 mmol) was treated as described in general procedure G, to give 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N'-4-

chlorobenzyl)-guanidinyl-3,14-dihydroxyindolo[2',3':6,7]morphinan (**107**) as the bistrifluoroacetic acid salt (0.077 g, 0.09 mmol, 74%); mp. 186-189 °C; Rf = 0.13 [$CH_3OH/CH_2Cl_2/NH_4OH$ (89:10:1)]; IR ν_{max}/cm (KBr) 3630-2475 (br, bonded OH and NH) and 1678 (br, C=N, NH and NH_2); ^1H NMR (400 MHz, $CDCl_3$) δ 0.48-0.59 [m, 2H, $NCH_2CH(CHHCHH)$], 0.73-0.91 [m, 2H, $NCH_2CH(CHHCHH)$], 1.09-1.22 [m, 1H, $NCH_2CH(CH_2CH_2)$], 5.71 [s, 1H, C(5)*H*], 6.64 [d, J=8.2 Hz, 1H, C(1)*H*], 6.68 [d, J=8.2 Hz, 1H, C(2)*H*], 6.98-7.02 (m, 1H, Ar*H*) and 7.28-7.46 [m, 6H, Ar*H*]; ^{13}C NMR (100 MHz, $CDCl_3$) δ 3.5 [$NCH_2CH(CH_2CH_2)$], 6.3 [$NCH_2CH(CH_2CH_2)$], 6.9 [$NCH_2CH(CH_2CH_2)$], 25.1 [*C*(10)], 29.7 [*C*(15)], 30.2 [*C*(8)], 45.3 [*C*(16)], 47.5 [quaternary *C*(13)], 48.0 (CH_2Ph), 58.9 [*C*(18)], 63.6 [*C*(9)], 73.5 [quaternary *C*(14)], 84.8 [*C*(5)], 109.9 (Ar), 113.7 (Ar), 118.0 (Ar), 119.2 (Ar), 120.5 (Ar), 122.2 (Ar), 122.4 (Ar), 126.6 (Ar), 128.3 (Ar), 129.6 (2 x Ar), 129.7 (2 x Ar), 130.1 (Ar), 132.3 (Ar), 134.4 (Ar), 136.4 (Ar), 138.0 (Ar), 141.8 (Ar), 144.5 (Ar) and 157.5 (*C*=NH); FAB$^+$MS *m/z* 596 [$(M+1)^+$, 100%]; HRMS (FAB) *m/z* 596.2416 $(M+1)^+$, $C_{34}H_{35}N_5O_3Cl$ requires 596.2428; Anal. ($C_{34}H_{34}N_5O_3Cl.2TFA.3H_2O$) requires C 51.97, H 4.82, N 7.97, found C 51.80, H 4.43, N 7.81.

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N'-4-nitrobenzyl)guanidinyl-3,14-dihydroxyindolo[2',3':6,7]morphinan (108)

1,3-Bis-BOC-1-(4'-nitrobenzyl-2-methyl-2-thiopseudourea (1.046 g, 2.47 mmol), 5'-amino-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-di-hydroxyindolo[2',3':6,7]morphinan (**120**) (0.530 g, 1.24 mmol), $HgCl_2$ (0.336 g, 1.24 mmol) and triethylamine (0.254 g, 0.35 ml, 2.51 mmol) were reacted according to general procedure F and stirred at 50 °C for 24 h, to give 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-bis-BOC-(N'-4-nitrobenzyl)guanidinyl-3,14-dihydroxyindolo[2',3':6,7]morphinan (0.426 g, 0.53 mmol, 43%); Rf = 0.52 [$CH_3OH/CH_2Cl_2/NH_4OH$ (89:10:1)]; ^1H NMR (400 MHz, $CDCl_3$) δ 0.09-0.21 [m, 2H, $NCH_2CH(CHHCHH)$], 0.49-0.61 [m, 2H, $NCH_2CH(CHHCHH)$], 0.80-0.94 [m, 1H, $NCH_2CH(CH_2CH_2)$], 1.36 [s, 9H, $C(CH_3)_3$], 1.44 [s, 9H, $C(CH_3)_3$], 5.60 [s, 1H, C(5)*H*], 6.39-6.53 [m, 2H, C(1)*H* and C(2)*H*], 6.69-7.40 (m, 4H, Ar*H*), 7.59-7.66 (m, 1H, Ar*H*), 7.88-7.98 (m, 1H, Ar*H*) and 8.03-8.12 [m, 1H, Ar*H*]; ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 3.7 [$NCH_2CH(CH_2CH_2)$], 4.0 [$NCH_2CH(CH_2CH_2)$], 9.3 [$NCH_2CH(CH_2CH_2)$], 23.0 [*C*(10)], 27.5 [$C(CH_3)_3$], 27.9 [$C(CH_3)_3$], 28.6

[*C*(15)], 31.4 [*C*(8)], 36.4 [*C*(16)], 43.5 (CH_2Ph), 47.9 [quaternary *C*(13)], 59.3 [*C*(18)], 62.1 [*C*(9)], 72.8 [quaternary *C*(14)], 83.2 [$C(CH_3)_3$], 84.9 [*C*(5)], 110.7 (Ar), 117.2 (Ar), 118.7 (Ar), 123.3 (2 x Ar), 124.9 (Ar), 127.4 (Ar), 128.8 (2 x Ar), 130.6 (Ar), 139.2 (Ar), 143.0 (Ar), 146.6 (Ar), 154.6 (Ar) and 162.7 (C=N); FAB^+MS *m/z* 807 [$(M+1)^+$, 100%], 707 (20) and 607 (10); HRMS (FAB) *m/z* 807.3731 $(M+1)^+$, $C_{44}H_{51}N_6O_9$ requires 807.3718.

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-bis-BOC-(N'-4-nitrobenzyl)guanidinyl-3,14-dihydroxyindolo[2',3':6,7]morphinan (0.058 g, 0.07 mmol) was treated as described in general procedure G, to give 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N'-4-nitrobenzyl)-guanidinyl-3,14-dihydroxyindolo[2',3':6,7]morphinan (**108**) as the bistrifluoroacetic acid salt (0.059 g, 0.07 mmol, 98%); mp. 206-208 °C; Rf = 0.13 [$CH_3OH/CH_2Cl_2/NH_4OH$ (89:10:1)]; IR ν_{max}/cm (KBr) 3700-2400 (br, bonded OH and NH) and 1677 (br, C=N, NH and NH_2); ^1H NMR (400 MHz, CD_3OD) δ 0.50-0.59 [m, 2H, $NCH_2CH(CHHCHH)$], 0.74-0.92 [m, 2H, $NCH_2CH(CHHCHH)$], 1.11-1.21 [m, 1H, $NCH_2CH(CH_2CH_2)$], 5.72 [s, 1H, C(5)*H*], 6.63 [d, J=8.2 Hz, 1H, C(1)*H*], 6.67 [d, J=8.2 Hz, 1H, C(2)*H*], 7.04 [dd, J_1=2.0 Hz, J_2=8.6 Hz, 1H, C(6')*H*], 7.33 [d, J=2.0 Hz, 1H, C(4')*H*], 7.45 [d, J=8.6 Hz, 1H, C(7')*H*], 7.55 [d, J=8.7 Hz, 2H, Ar*H*] and 8.24 [d, J=8.7 Hz, 2H, Ar*H*]; ^{13}C NMR (100 MHz, CD_3OD) δ 4.3 [$NCH_2CH(CH_2CH_2)$], 7.1 [$NCH_2CH(CH_2CH_2)$], 7.7 [$NCH_2CH(CH_2CH_2)$], 25.9 [*C*(10)], 30.6 [*C*(15)], 31.1 [*C*(8)], 46.1 [*C*(16)], 48.4 [quaternary *C*(13)], 48.9 (CH_2Ph), 59.8 [*C*(18)], 67.6 [*C*(9)], 74.4 [quaternary *C*(14)], 85.6 [*C*(5)], 110.8 (Ar), 114.6 (Ar), 118.9 (Ar), 120.1 (Ar), 121.3 (Ar), 123.0 (Ar), 123.0 (Ar), 125.5 (2 x Ar), 127.4 (Ar), 129.2 (Ar), 129.7 (2 x Ar), 130.9 (Ar), 133.2 (Ar), 138.9 (Ar), 142.7 (Ar), 145.4 (Ar), 146.2 (Ar), 149.5 (Ar) and 158.6 (*C*=NH); FAB^+MS *m/z* 607 [$(M+1)^+$, 100%]; HRMS (FAB) *m/z* 607.2677 $(M+1)^+$, $C_{34}H_{35}N_6O_5$ requires 607.2669; Anal. ($C_{34}H_{34}N_6O_5.2TFA.3.25H_2O$) requires C 51.09, H 4.80, N 9.41, found C 50.60, H 4.30, N 9.15.

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N'-4-aminobenzyl)guanidinyl-3,14-dihydroxyindolo[2',3':6,7]morphinan (109)

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-bis-BOC-(N'-4-nitrobenzyl)guanidinyl-3,14-dihydroxyindolo[2',3':6,7]morphinan (0.113 g, 0.14 mmol) was dissolved in CH_3OH (30 ml) and

cyclohexene (30 ml). To this was added 3 drops of triethylamine and a catalytic amount of Raney Nickel catalyst. The biphasic solution was stirred at RT for 5 h, filtered through a cellite pad, and concentrated to yield the crude product, which was then purified by column chromatography [$CH_3OH/CH_2Cl_2/NH_4OH$ (89:10:1)] to afford 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-bis-BOC-(N'-4-aminobenzyl)guanidinyl-3,14-dihydroxyindolo[2',3':6,7]-morphinan (**126**) (0.068 g, 0.09 mmol, 63%); Rf = 0.47 [$CH_3OH/CH_2Cl_2/NH_4OH$ (89:10:1)]; ^1H NMR ($CDCl_3$) δ 0.25-1.67 [m; 23H; $NCH_2CH(CHHCHH)$, $NCH_2CH(CHHCHH)$, $NCH_2CH(CH_2CH_2)$, $C(CH_3)_3$, $C(CH_3)_3$], 5.72 [s, 1H, C(5)*H*] and 6.68-7.43 [m, 9H, Ar*H*]; ^{13}C NMR (67.8 MHz, CD_3OD) δ 4.6 [$NCH_2CH(CH_2CH_2)$], 4.9 [$NCH_2CH(CH_2CH_2)$], 10.5 [$NCH_2CH(CH_2CH_2)$], 24.4 [*C*(10)], 28.5 [*C*(15)], 28.7 [$C(CH_3)_3$], 30.1 [*C*(8)], 32.8 [*C*(16)], 45.0 (benzylic CH_2), 48.3 [quaternary *C*(13)], 60.5 [*C*(18)], 63.7 [*C*(9)], 74.4 [quaternary *C*(14)], 81.3 [$C(CH_3)_3$], 83.7 [*C*(5)], 111.9 (Ar), 112.9 (Ar), 113.5 (Ar), 116.5 (2 x Ar), 118.5 (Ar), 119.5 (Ar), 120.2 (Ar), 126.3 (Ar), 127.3 (Ar), 128.3 (Ar), 131.5 (2 x Ar), 132.4 (2 x Ar), 137.0 (Ar), 141.3 (Ar), 144.8 (Ar), 148.7 (Ar) and 154.6 (*C*=N); FAB^+MS *m/z* 777 [$(M+1)^+$, 100%], 677 (55) and 576 (55); HRMS (FAB) *m/z* 777.3992 $(M+1)^+$, $C_{44}H_{53}N_6O_7$ requires 777.3976.

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-bis-BOC-(N'-4-aminobenzyl)guanidinyl-3,14-dihydroxyindolo[2',3':6,7]morphinan (**127**) (0.036 g, 0.05 mmol) was treated as described in general procedure G, to give 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N'-4-aminobenzyl)-guanidinyl-3,14-dihydroxyindolo[2',3':6,7]morphinan (**109**) as the tristrifluoroacetic acid salt (0.033 g, 0.04 mmol, 78%); mp. >250 °C; Rf = 0.10 [$CH_3OH/CH_2Cl_2/NH_4OH$ (89:10:1)]; IR ν_{max}/cm (KBr) 3680-2420 (br, bonded OH and NH) and 1677 (br, C=N, NH and NH_2); ^1H NMR (400 MHz, CD_3OD) δ 0.49-0.60 [m, 2H, $NCH_2CH(CHHCHH)$], 0.74-0.92 [m, 2H, $NCH_2CH(CHHCHH)$], 1.12-1.20 [m, 1H, $NCH_2CH(CH_2CH_2)$], 5.72 [s, 1H, C(5)*H*], 6.64 [d, J=8.2 Hz, 1H, C(1)*H*], 6.67 [d, J=8.2 Hz, 1H, C(2)*H*], 7.01 [dd, J_1=1.8 Hz, J_2=8.4 Hz, 1H, C(6')*H*], 7.14 [d, J=8.2 Hz, 2H, Ar*H*], 7.32 [d, J=8.2 Hz, 2H, Ar*H*], 7.34 [d, J=1.8 Hz, 1H, C(4')*H*] and 7.44 [d, J=8.4 Hz, 1H, C(7')*H*]; ^{13}C NMR (67.8 MHz, CD_3OD) δ 3.7 [$NCH_2CH(CH_2CH_2)$], 6.5 [$NCH_2CH(CH_2CH_2)$], 7.1 [$NCH_2CH(CH_2CH_2)$], 25.3 [*C*(10)], 30.0 [*C*(15)], 30.5 [*C*(8)], 45.9 [*C*(16)], 47.9 [quaternary *C*(13)], 48.3 (benzylic CH_2), 59.2 [*C*(18)], 63.9 [*C*(9)], 73.9 [quaternary *C*(14)], 85.2 [*C*(5)], 110.4 (Ar), 114.2 (Ar), 118.6 (Ar), 119.7 (Ar), 120.9 (Ar), 121.4 (Ar), 122.7

(Ar), 122.9 (Ar), 127.2 (Ar), 128.9 (Ar), 130.1 (Ar), 130.6 (Ar), 132.8 (Ar), 138.6 (Ar), 142.4 (Ar), 145.1 (Ar) and 158.0 (*C*=NH); FAB$^+$MS *m/z* 577 [$(M+1)^+$, 65%] and 472 (100); HRMS (FAB) *m/z* 577.2922 $(M+1)^+$, $C_{34}H_{37}N_6O_3$ requires 577.2927; Anal. ($C_{34}H_{36}N_6O_3$.3TFA.1H_2O) requires C 51.29, H 4.41, N 8.97, found C 51.40, H 4.55, N 8.78.

N- *tert*-butoxycarbonyl -N,N'-dibutylthiourea

N,N'-Dibutylthiourea (4.05 g, 21.56 mmol), NaH (1.75 g, 60% in oil, 43.75 mmol) and $(BOC)_2O$ (5.41 g, 24.80 mmol) were reacted according to general procedure I, to give N-*tert*-butoxycarbonyl-N,N'-dibutylthiourea (3.52 g, 12.22 mmol, 57%); R_f = 0.59 [EtOAc/hexane (1:3)]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.78-0.84 (m, 6H, 2 x C*H*$_3$), 1.17-1.31 [m, 4H, 2 x C*H*$_2$CH$_3$], 1.40 [s, 9H, C(C*H*$_3$)$_3$], 1.47-1.55 [m, 4H, 2 x C*H*$_2$CH$_2$CH$_3$], 3.44-3.51 (m, 2H, NHC*H*$_2$), 4.10-4.18 (m, 2H, CONC*H*$_2$) and 10.70 (br s, 1H, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.5 (*C*H$_3$), 13.6 (*C*H$_3$), 19.7 (*C*H$_2$CH$_3$), 20.0 (*C*H$_2$CH$_3$), 27.7 [C(*C*H$_3$)$_3$], 30.0 (*C*H$_2$CH$_2$CH$_3$), 30.4 (*C*H$_2$CH$_2$CH$_3$) 46.4 (HN*C*H$_2$), 48.7 (CON*C*H$_2$), 83.3 [*C*(CH$_3$)$_3$], 154.7 (C=O) and 182.5 (*C*=S); FAB$^+$MS *m/z* 289 [$(M+1)^+$, 45%] and 233 (100).

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N',N''-dibutyl)guanidinylethyl-3,14-dihydroxyindolo[2',3':6,7]morphinan (113)

N-BOC-N,N'-dibutylthiourea (0.09 g, 0.32 mmol), 5'-aminoethyl-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-dihydroxyindolo[2',3':6,7]morphinan (**76**) (0.112 g, 0.25 mmol), $HgCl_2$ (0.087 g, 0.32 mmol) and triethylamine (0.025 g, 0.035 ml, 0.25 mmol) were reacted according to general procedure F and stirred at 50 °C for 5 h, to give 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-BOC-(N',N''-dibutyl)guanidinylethyl-3,14-dihydroxyindolo[2',3':6,7]-morphinan (0.033 g, 0.05 mmol, 19%); Rf = 0.33 [$CH_3OH/CH_2Cl_2/NH_4OH$ (89:10:1)]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.10-0.19 [m, 2H, NCH$_2$CH(C*H*HC*H*H)], 0.50-0.61 [m, 2H, NCH$_2$CH(CH*H*CH*H*)], 0.85-0.92 [m, 1H, NCH$_2$C*H*(CH$_2$CH$_2$)], 1.42 [s, 9H, C(C*H*$_3$)$_3$], 5.60 [s, 1H, C(5)*H*], 6.45 [d, J=7.8 Hz, 1H, C(1)*H*], 6.61 [d, J=7.8 Hz, 1H, C(2)*H*], 6.74-6.88 [m, 1H, Ar*H*] and 6.97-7.16 [m, 2H, Ar*H*]; ^{13}C NMR (100 MHz, $CDCl_3$) δ 3.9 [NCH$_2$CH(*C*H$_2$CH$_2$)], 4.2

[$NCH_2CH(CH_2CH_2)$], 9.5 [$NCH_2CH(CH_2CH_2)$], 13.6 (2 x CH_3), 13.7 (2 x CH_2), 19.9 (2 x CH_2), 21.2 (2 x CH_2), 23.2 [C(10)], 28.1 [$C(CH_3)_3$], 28.9 (CH_2), 30.4 (CH_2), 30.6 (CH_2), 31.4 (CH_2), 43.7 [C(16)], 48.0 [quaternary C(13)], 59.5 [C(18)], 62.4 [C(9)], 72.6 [quaternary C(14)], 84.9 [C(5)], 110.6 (Ar), 111.2 (Ar), 117.4 (Ar), 118.4 (Ar), 118.6 (Ar), 123.4 (Ar), 124.4 (Ar), 126.8 (Ar), 129.7 (Ar), 130.6 (Ar), 136.0 (Ar), 139.5 (Ar) and 143.1 (Ar); FAB^+MS *m/z* 712 [$(M+1)^+$, 45%] and 612 (100).

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-BOC-(N',N''-dibutyl)guanidinylethyl-3,14-dihydroxyindolo[2',3':6,7]morphinan (0.033 g, 0.05 mmol) was treated as described in general procedure G, to give 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N',N''-dibutyl)-guanidinylethyl-3,14-dihydroxyindolo[2',3':6,7]morphinan (**113**) as the bistrifluoroacetic acid salt (0.033 g, 0.04 mmol, 85%); mp. 160-162 °C; Rf = 0.17 [$CH_3OH/CH_2Cl_2/NH_4OH$ (89:10:1)]; IR ν_{max}/cm (KBr) 3670-2470 (br, bonded OH and NH) and 1678 (br, C=N, NH and NH_2); ^1H NMR (270 MHz, CD_3OD) δ 0.39-0.49 [m, 2H, $NCH_2CH(CHHCHH)$], 5.60 [s, 1H, C(5)*H*], 6.52-6.61 [m, 2H, C(1)*H* and C(2)*H*], 6.88-6.95 [m, 1H, C(6')*H*], 7.16 [s, 1H, C(4')*H*] and 7.18-7.24 [m, 1H, C(7')*H*]; ^{13}C NMR (67.8 MHz, CD_3OD) δ 3.3 [$NCH_2CH(CH_2CH_2)$], 6.2 [$NCH_2CH(CH_2CH_2)$], 6.8 [$NCH_2CH(CH_2CH_2)$], 13.8 (CH_3), 20.7 (CH_2), 25.0 [C(10)], 29.8 (CH_2), 30.3 (CH_2), 31.8 (CH_2), 36.2 (CH_2), 42.1 (CH_2), 44.4 [C(16)], 47.5 [quaternary C(13)], 58.8 [C(18)], 63.6 [C(9)], 73.7 [quaternary C(14)], 85.1 [C(5)], 109.2 (Ar), 112.7 (Ar), 119.3 (Ar), 119.6 (Ar), 120.4 (Ar), 122.4 (Ar), 124.7 (Ar), 128.3 (Ar), 129.8 (Ar), 130.3 (Ar), 131.0 (Ar), 138.0 (Ar), 142.1 (Ar), 144.8 (Ar) and 155.8 (C=N); FAB^+MS *m/z* 612 [$(M+1)^+$, 100%]; HRMS (FAB) *m/z* 612.3901 $(M+1)^+$, $C_{37}H_{50}N_5O_3$ requires 612.3914; Anal. ($C_{37}H_{49}N_5O_3.2TFA.1H_2O$) requires C 57.40, H 6.23, N 8.16, found C 57.60, H 5.99, N 7.91.

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N',N''-dibutyl)guanidinyl-3,14-dihydroxyindolo[2',3':6,7]morphinan (114)

N-BOC-N,N'-dibutylthiourea (0.095 g, 0.33 mmol), 5'-amino-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-dihydroxyindolo[2',3':6,7]-morphinan (**120**) (0.087 g, 0.20 mmol), $HgCl_2$ (0.090 g, 0.33 mmol) and triethylamine (0.026 g, 0.036 ml, 0.26 mmol) were reacted

according to general procedure F and stirred at 50 °C for 48 h, to give 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-BOC-(N',N''-dibutyl)guanidinyl-3,14-dihydroxyindolo[2',3':6,7]-morphinan (0.027 g, 0.04 mmol, 20%); Rf = 0.52 [$CH_3OH/CH_2Cl_2/NH_4OH$ (89:10:1)]; ^1H NMR (400 MHz, $CDCl_3$) δ 0.15-0.21 [m, 2H, $NCH_2CH(CHHCHH)$], 0.55-0.62 [m, 2H, $NCH_2CH(CHHCHH)$], 1.49 [s, 9H, $C(CH_3)_3$], 5.62 [s, 1H, C(5)*H*], 6.43-7.10 (m, 5H, Ar*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 4.0 [$NCH_2CH(CH_2CH_2)$], 4.1 [$NCH_2CH(CH_2CH_2)$], 9.6 [$NCH_2CH(CH_2CH_2)$], 13.9 (CH_3), 20.2 (CH_2), 23.3 [*C*(10)], 28.5 [$C(CH_3)_3$], 28.6 (CH_2), 29.0 (CH_2), 30.8 (CH_2), 31.6 (CH_2), 43.8 [*C*(16)], 48.1 [quaternary *C*(13)], 59.6 [*C*(18)], 63.1 [*C*(9)], 72.7 [quaternary *C*(14)], 81.0 [quaternary $C(CH_3)_3$], 85.2 [*C*(5)], 110.9 (Ar), 111.5 (Ar), 117.4 (Ar), 118.0 (Ar), 118.6 (Ar), 124.6 (Ar), 127.3 (Ar), 127.4 (Ar), 130.6 (Ar), 134.1 (Ar), 139.3 (Ar) and 143.2 (Ar); FAB^+MS *m/z* 684 [$(M+1)^+$, 100%] and 584 (40); HRMS (FAB) *m/z* 684.4128 $(M+1)^+$, $C_{40}H_{54}N_5O_5$ requires 684.4125.

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-BOC-(N',N''-dibutyl)guanidinyl-3,14-di-hydroxyindolo[2',3':6,7]morphinan (0.020 g, 0.03 mmol) was treated as described in general procedure G, to give 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N',N''-dibutyl)-guanidinyl-3,14-dihydroxyindolo [2',3':6,7]morphinan (**114**) as the bistrifluoroacetic acid salt (0.020 g, 0.03 mmol, 86%); mp. 196-199 °C; Rf = 0.09 [$CH_3OH/CH_2Cl_2/NH_4OH$ (89:10:1)]; IR ν_{max}/cm (KBr) 3650-2680 (br, bonded OH and NH) and 1678 (br, C=N, NH and NH_2); ^1H NMR (400 MHz, CD_3OD) δ 0.52-0.59 [m, 2H, $NCH_2CH(CHHCHH)$], 5.74 [s, 1H, C(5)*H*], 6.64 [d, J=8.2 Hz, 1H, C(1)*H*], 6.67 [d, J=8.2 Hz, 1H, C(2)*H*], 7.00 [dd, J_1=8.6 Hz, J_2=2.1 Hz, 1H, C(6')*H*], 7.31 [d, J=2.1 Hz, 1H, C(4')*H*] and 7.46 [d, J=8.6 Hz, 1H, C(7')*H*]; ^{13}C NMR (67.8 MHz, CD_3OD) δ 3.7 [$NCH_2CH(CH_2CH_2)$], 6.5 [$NCH_2CH(CH_2CH_2)$], 7.1 [$NCH_2CH(CH_2CH_2)$], 14.3 (CH_3), 15.7 (CH_2), 21.2 (CH_2), 25.3 [*C*(10)], 30.0 (CH_2), 30.6 (CH_2), 32.4 (CH_2), 42.8 [*C*(16)], 47.9 [quaternary *C*(13)], 59.2 [*C*(18)], 63.9 [*C*(9)], 73.9 [quaternary *C*(14)], 85.2 [*C*(5)], 110.3 (Ar), 114.2 (Ar), 118.7 (Ar), 119.7 (Ar), 120.9 (Ar), 122.9 (Ar), 127.4 (Ar), 129.0 (Ar), 130.6 (Ar), 132.9 (Ar), 138.5 (Ar), 142.5 (Ar), 145.1 (Ar), 147.0 (Ar) and 156.6 (C=N); FAB^+MS *m/z* 584 [$(M+1)^+$, 100%]; HRMS (FAB) *m/z* 584.3603 $(M+1)^+$, $C_{35}H_{46}N_5O_3$ requires 584.3601.

N,N'-Dipropylthiourea

Propylamine (1.16 g, 19.67 mmol), calcium carbonate (1.97 g, 19.67 mmol) and thiophosgene (4.52 g, 3 ml, 39.34 mmol) were reacted according to general procedure H, to give propylisothiocyanate; R_f = 0.72 [EtOAc/hexane (1:1)]. Propylisothiocyanate (0.48 g, 4.75 mmol) and propylamine (0.28 g, 4.75 mmol) were then reacted to give N,N'-dipropylthiourea (0.35 g, 2.19 mmol, 46%); R_f = 0.37 [EtOAc/hexane (1:1)]; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.92 (t, J=7.3 Hz, 6H, 2 x C*H*$_3$), 1.58 [q, J=7.3 Hz, 4H, 2 x C*H*$_2$CH$_3$), 3.36 [br s, 4H, 2 x NC*H*$_2$] and 6.09 [br s, 2H, 2 x N*H*]; ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 11.3 (*C*H$_3$), 22.2 (*C*H$_2$CH$_3$), 46.0 (NCH$_2$) and 181.3 (*C*=S); FAB$^+$MS *m/z* 161 [(M+1)$^+$, 100%]; HRMS (FAB) *m/z* 161.1111 (M+1)$^+$, $C_7H_{17}N_2S$ requires 161.1112.

N-*tert*-butoxycarbonyl-N,N'-dipropylthiourea

N,N'-Dipropylthiourea (0.26 g, 1.64 mmol), NaH (0.08 g, 60% in oil, 3.28 mmol) and $(BOC)_2O$ (0.41 g, 1.88 mmol) were reacted according to general procedure I, to give N-*tert*-butoxycarbonyl-N,N'-dipropylthiourea (0.18 g, 0.71 mmol, 43%); R_f = 0.70 [EtOAc/hexane (1:3)]; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.79-0.95 (m, 6H, 2 x C*H*$_3$), 1.34-1.48 [m, 9H, C(C*H*$_3$)$_3$], 1.49-1.69 [m, 4H, 2 x C*H*$_2$CH$_3$], 3.45-3.61 (m, 2H, NHC*H*$_2$), 4.07-4.29 (m, 2H, CONC*H*$_2$) and 10.80 (br s, 1H, N*H*); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 11.0 (*C*H$_3$), 11.6 (*C*H$_3$), 21.4 (*C*H$_2$CH$_3$), 21.9 (*C*H$_2$CH$_3$), 30.2 [C(*C*H$_3$)$_3$], 48.7 (HN*C*H$_2$), 50.7 (CON*C*H$_2$), 83.7 [*C*(CH$_3$)$_3$], 155.2 (C=O) and 183.2 (*C*=S); FAB$^+$MS *m/z* 261 [(M+1)$^+$, 40%]; HRMS (FAB) *m/z* 261.1628 (M+1)$^+$, $C_{12}H_{25}N_2O_2S$ requires 261.1637.

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N',N''-dipropyl)guanidinyl-3,14-dihydroxyindolo[2',3':6,7]morphinan (115)

N-BOC-N,N'-dipropylthiourea (0.100 g, 0.39 mmol), 5'-amino-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-dihydroxyindolo[2',3':6,7]-morphinan (**120**) (0.113 g, 0.26 mmol), $HgCl_2$ (0.105 g, 0.39 mmol) and triethylamine (0.030 g, 0.042 ml, 0.30 mmol) were reacted

according to general procedure F and stirred at 50 °C for 48 h, to give 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-BOC-(N',N''-dipropyl)guanidinyl-3,14-dihydroxyindolo[2',3':6,7]-morphinan (0.068 g, 0.10 mmol, 39%); Rf = 0.50 [$CH_3OH/CH_2Cl_2/NH_4OH$ (89:10:1)]; ^1H NMR (270 MHz, $CDCl_3$) δ 0.12-0.20 [m, 2H, $NCH_2CH(CHHCHH)$], 0.52-0.61 [m, 2H, $NCH_2CH(CHHCHH)$], 1.48 [s, 9H, $C(CH_3)_3$], 5.61 [s, 1H, C(5)*H*], 6.41-7.09 (m, 5H, Ar*H*); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 3.8 [$NCH_2CH(CH_2CH_2)$], 3.9 [$NCH_2CH(CH_2CH_2)$], 9.4 [$NCH_2CH(CH_2CH_2)$], 11.4 (CH_3), 21.8 (CH_2), 23.1 [*C*(10)], 28.3 [$C(CH_3)_3$], 28.8 (CH_2), 31.4 (CH_2), 43.6 (CH_2), 44.7 [*C*(16)], 47.9 [quaternary *C*(13)], 59.5 [*C*(18)], 62.4 [*C*(9)], 72.7 [quaternary *C*(14)], 81.1 [quaternary $C(CH_3)_3$], 85.1 [*C*(5)], 110.8 (Ar), 117.6 (Ar), 118.6 (Ar), 124.6 (Ar), 127.3 (Ar), 129.6 (Ar), 130.6 (Ar), 134.2 (Ar), 139.5 (Ar), 143.3 (Ar) and 154.1 (*C*=N); FAB^+MS *m/z* 656 [$(M+1)^+$, 100%] and 556 (50); HRMS (FAB) *m/z* 656.3817 $(M+1)^+$, $C_{38}H_{50}N_5O_5$ requires 656.3812.

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-BOC-(N',N''-dipropyl)guanidinyl-3,14-di-hydroxyindolo[2',3':6,7]morphinan (0.025 g, 0.04 mmol) was treated as described in general procedure G, to give 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N',N''-dipropyl)-guanidinyl-3,14-dihydroxyindolo[2',3':6,7]morphinan (**115**) as the bistrifluoroacetic acid salt (0.023 g, 0.03 mmol, 78%); mp. 174-175 °C; Rf = 0.09 [$CH_3OH/CH_2Cl_2/NH_4OH$ (89:10:1)]; IR ν_{max}/cm (KBr) 3700-2730 (br, bonded OH and NH) and 1678 (br, C=N, NH and NH_2); ^1H NMR (270 MHz, CD_3OD) δ 0.41-0.52 [m, 2H, $NCH_2CH(CHHCHH)$], 5.64 [s, 1H, C(5)*H*], 6.53-6.62 [m, 2H, C(1)*H* and C(2)*H*], 6.91 [dd, J_1=8.6 Hz, J_2=2.2 Hz, 1H, C(6')*H*], 7.23 [d, J=2.2 Hz, 1H, C(4')*H*] and 7.37 [d, J=8.6 Hz, 1H, C(7')*H*]; ^{13}C NMR (67.8 MHz, CD_3OD) δ 3.7 [$NCH_2CH(CH_2CH_2)$], 6.5 [$NCH_2CH(CH_2CH_2)$], 7.1 [$NCH_2CH(CH_2CH_2)$], 11.6 (CH_3), 23.6 [*C*(10)], 25.3 (CH_2), 30.0 (CH_2), 30.6 (CH_2), 44.6 [*C*(16)], 47.9 [quaternary *C*(13)], 59.2 [*C*(18)], 63.9 [*C*(9)], 73.9 [quaternary *C*(14)], 85.2 [*C*(5)], 110.3 (Ar), 114.2 (Ar), 118.8 (Ar), 119.7 (Ar), 120.9 (Ar), 122.9 (Ar), 123.0 (Ar), 127.3 (Ar), 129.0 (Ar), 130.6 (Ar), 132.9 (Ar), 138.5 (Ar), 142.5 (Ar), 145.1 (Ar), and 156.6 (C=N); FAB^+MS *m/z* 556 [$(M+1)^+$, 100%]; HRMS (FAB) *m/z* 556.3287 $(M+1)^+$, $C_{33}H_{42}N_5O_3$ requires 556.3288.

N-Propyl-N'-cyclopropylmethylthiourea

Propylamine (1.16 g, 19.67 mmol), calcium carbonate (1.97 g, 19.67 mmol) and thiophosgene (4.52 g, 3 ml, 39.34 mmol) were reacted according to general procedure H, to give propylisothiocyanate; R_f = 0.72 [EtOAc/hexane (1:1)]. Propylisothiocyanate (0.44 g, 4.38 mmol) and aminomethylcyclopropane (0.31 g, 4.38 mmol) were then reacted to give N-propyl-N'-cyclopropylmethylthiourea (0.42 g, 2.44 mmol, 56%); R_f = 0.38 [EtOAc/hexane (1:1)]; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.21-0.30 [m, 2H, $NCH_2CH(CHHCHH)$], 0.52-0.62 [m, 2H, $NCH_2CH(CHHCHH)$], 0.97 (t, J=7.3 Hz, 3H, CH_3), 1.02-1.13 [m, 1H, $NCH_2CH(CH_2CH_2)$], 1.64 [q, J=7.3 Hz, 2H, CH_2CH_3), 3.30-3.41 [m, 4H, 2 x NCH_2] and 6.24 [br s, 2H, 2 x NH]; ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 3.5 [$NCH_2CH(CH_2CH_2)$] and [$NCH_2CH(CH_2CH_2)$], 10.0 [$NCH_2CH(CH_2CH_2)$], 11.3 (CH_3), 22.1 (CH_2CH_3), 45.9 (NCH_2), 49.4 (NCH_2CH) and 181.0 (C=S); FAB$^+$MS *m/z* 173 [$(M+1)^+$, 100%]; HRMS (FAB) *m/z* 173.1116 $(M+1)^+$, $C_8H_{17}N_2S$ requires 173.1112.

N-*tert*-butoxycarbonyl-N-propyl-N'-cyclopropylmethylthiourea

N-Propyl-N'-cyclopropylmethylthiourea (0.41 g, 2.38 mmol), NaH (0.19 g, 60% in oil, 4.75 mmol) and $(BOC)_2O$ (0.60 g, 2.73 mmol) were reacted according to general procedure I, to give N-*tert*-butoxycarbonyl-N-propyl,N'-cyclopropylmethylthiourea (0.30 g, 1.09 mmol, 46%); R_f = 0.66 [EtOAc/hexane (1:3)]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.22-0.49 [m, 4H, $NCH_2CH(CHHCHH)$ and $NCH_2CH(CHHCHH)$], 0.51-0.57 [m, 1H, $NCH_2CH(CH_2CH_2)$], 0.88 (t, J=7.4 Hz, 3H, CH_3), 1.51 [s, 9H, $C(CH_3)_3$], 1.65 [q, J=7.4 Hz, 2H, CH_2CH_3], 3.39-3.60 (m, 2H, $NHCH_2$), 4.16-4.30 (m, 2H, $CONCH_2$) and 10.86 (br s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 4.0 [$NCH_2CH(CH_2CH_2)$], 4.1 [$NCH_2CH(CH_2CH_2)$], 11.0 [$NCH_2CH(CH_2CH_2)$], 11.7 (CH_3), 22.3 (CH_2CH_3), 28.4 [$C(CH_3)_3$], 51.1 ($HNCH_2$), 57.2 ($CONCH_2$), 83.3 [$C(CH_3)_3$], 151.7 (C=O) and 183.0 (C=S); FAB$^+$MS *m/z* 273 [$(M+1)^+$, 25%] and 217 (85); HRMS (FAB) *m/z* 273.1644 $(M+1)^+$, $C_{13}H_{25}N_2O_2S$ requires 273.1637.

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N'-propyl,N''-cyclopropylmethyl-thiourea)guanidinyl-3,14-di-hydroxyindolo[2',3':6,7]morphinan (116)

N-BOC-N-propyl,N'-cyclopropylmethylthiourea (0.152 g, 0.56 mmol), 5'-amino-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-dihydroxyindolo[2',3':6,7]-morphinan (**120**) (0.120 g, 0.28 mmol), $HgCl_2$ (0.152 g, 0.56 mmol) and triethylamine (0.028 g, 0.040 ml, 0.28 mmol) were reacted according to general procedure F and stirred at 50 °C for 48 h, to give 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-BOC-(N'-propyl,N''-cyclopropylmethyl)-guanidinyl-3,14-dihydroxyindolo[2',3':6,7]morphinan (0.062 g, 0.09 mmol, 33%); Rf = 0.61 [$CH_3OH/CH_2Cl_2/NH_4OH$ (89:10:1)]; ^1H NMR (270 MHz, $CDCl_3$) δ 0.05-0.10 [m, 2H, $NCH_2CH(CHHCHH)$], 0.11-0.20 [m, 2H, $NCH_2CH(CHHCHH)$], 0.35-0.44 [m, 2H, $NCH_2CH(CHHCHH)$], 0.46-0.58 [m, 2H, $NCH_2CH(CHHCHH)$], 0.73-0.83 [m, 1H, $NCH_2CH(CH_2CH_2)$], 0.84-0.98 [m, 1H, $NCH_2CH(CH_2CH_2)$], 1.47 [s, 9H, $C(CH_3)_3$], 5.60 [s, 1H, C(5)*H*], 6.38-7.10 (m, 5H, Ar*H*); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 3.3 [$NCH_2CH(CH_2CH_2)$], 3.4 [$NCH_2CH(CH_2CH_2)$], 3.8 [$NCH_2CH(CH_2CH_2)$], 3.9 [$NCH_2CH(CH_2CH_2)$], 9.4 [$NCH_2CH(CH_2CH_2)$], 10.5 [$NCH_2CH(CH_2CH_2)$], 11.4 (CH_3), 21.8 (CH_2), 23.1 [C(10)], 28.3 [$C(CH_3)_3$], 28.8 (CH_2), 31.4 (CH_2), 43.6 (CH_2), 47.9 [quaternary C(13)], 59.5 [C(18)], 62.4 [C(9)], 72.7 [quaternary C(14)], 81.0 [quaternary $C(CH_3)_3$], 85.0 [C(5)], 110.7 (Ar), 111.7 (Ar), 117.5 (Ar), 117.9 (Ar), 118.5 (Ar), 124.5 (Ar), 127.3 (Ar), 129.6 (Ar), 130.6 (Ar), 134.2 (Ar), 139.6 (Ar), 143.4 (Ar), 153.9 (Ar) and 154.1 (C=N); FAB^+MS *m/z* 668 [$(M+1)^+$, 100%] and 568 (30); HRMS (FAB) *m/z* 668.3819 $(M+1)^+$, $C_{39}H_{50}N_5O_5$ requires 668.3812.

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-BOC-(N'-propyl,N''-cyclopropylmethyl)-guanidinyl-3,14-dihydroxyindolo[2',3':6,7]morphinan (0.046 g, 0.07 mmol) was treated as described in general procedure G, to give 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N'-propyl,N''-cyclopropylmethyl)guanidinyl-3,14-dihydroxyindolo[2',3':6,7]morphinan (**116**) as the bistrifluoroacetic acid salt (0.039 g, 0.07 mmol, 99%); mp. 177-178 °C; Rf = 0.24 [$CH_3OH/CH_2Cl_2/NH_4OH$ (89:10:1)]; IR ν_{max}/cm (KBr) 3700-2620 (br, bonded OH and NH) and 1678 (br, C=N, NH and NH_2); ^1H NMR (400 MHz, CD_3OD) δ 0.12-0.21 [m, 2H, $NCH_2CH(CHHCHH)$], 5.65 [s, 1H, C(5)*H*], 6.56 [d, J=8.2 Hz, 1H, C(1)*H*], 6.59 [d, J=8.2 Hz, 1H,

C(2)*H*], 6.93 [dd, J_1=8.6 Hz, J_2=1.9 Hz, 1H, C(6')*H*], 7.25 [d, J=1.9 Hz, 1H, C(4')*H*] and 7.38 [d, J=8.6 Hz, 1H, C(7')*H*]; ^{13}C NMR (67.8 MHz, CD_3OD) δ 3.7 [$NCH_2CH(CH_2CH_2)$], 4.2 [$NCH_2CH(CH_2CH_2)$], 6.5 [$NCH_2CH(CH_2CH_2)$], 7.1 [$NCH_2CH(CH_2CH_2)$], 11.5 [$NCH_2CH(CH_2CH_2)$], 11.7 (CH_3), 23.6 [*C*(10)], 25.3 (CH_2), 30.0 (CH_2), 30.5 (CH_2), 44.6 [*C*(16)], 47.6 (CH_2), 47.9 [quaternary *C*(13)], 59.2 [*C*(18)], 63.9 [*C*(9)], 73.9 [quaternary *C*(14)], 85.2 [*C*(5)], 110.3 (Ar), 114.2 (Ar), 118.6 (Ar), 119.7 (Ar), 120.9 (Ar), 122.8 (Ar), 122.9 (Ar), 127.4 (Ar), 129.0 (Ar), 130.6 (Ar), 132.9 (Ar), 138.5 (Ar), 142.4 (Ar), 145.1 (Ar) and 156.6 (C=N); FAB$^+$MS *m/z* 568 [$(M+1)^+$, 100%]; HRMS (FAB) *m/z* 568.3275 $(M+1)^+$, $C_{34}H_{42}N_5O_3$ requires 568.3288; Anal. ($C_{34}H_{41}N_5O_3$.2TFA.$2H_2O$) requires C 54.87, H 5.70, N 8.42, found C 54.90, H 5.44, N 8.12.

N-Benzyl-N'-cyclopropylmethylthiourea

Benzylamine (2.11 g, 2.15 ml, 19.67 mmol), calcium carbonate (1.97 g, 19.67 mmol) and thiophosgene (4.52 g, 3 ml, 39.34 mmol) were reacted according to general procedure H, to give benzylisothiocyanate. Benzylisothiocyanate (0.90 g, 6.02 mmol) and aminomethylcyclopropane (0.43 g, 5.99 mmol) were then reacted to give N-benzyl-N'-cyclopropylmethylthiourea (0.62 g, 2.82 mmol, 47%); R_f = 0.39 [EtOAc/hexane (1:1)]; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.00-0.14 [m, 2H, $NCH_2CH(CHHCHH)$], 0.26-0.46 [m, 2H, $NCH_2CH(CHHCHH)$], 0.75-0.90 [m, 1H, $NCH_2CH(CH_2CH_2)$], 3.11 (br s, 2H, CH_2), 4.50 (br s, 2H, CH_2Ph), 6.33 (br s, 1H, N*H*), 6.56 (br s, 1H, N*H*) and 7.11-7.26 (m, 5H, Ar*H*); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 3.3 [$NCH_2CH(CH_2CH_2)$] and [$NCH_2CH(CH_2CH_2)$], 9.8 [$NCH_2CH(CH_2CH_2)$], 48.1 (CH_2), 49.3 (CH_2), 127.3 [*C*(2) and *C*(6)], 127.5 [*C*(4)], 128.5 [*C*(3) and *C*(5)], 137.0 [quaternary *C*(1)] and 181.2 (*C*=S); FAB$^+$MS *m/z* 221 [$(M+1)^+$, 100%].

N-*tert*-butoxycarbonyl-N-benzyl-N'-cyclopropylmethylthiourea

N-Benzyl-N'-cyclopropylmethylthiourea (0.30 g, 1.36 mmol), NaH (0.11 g, 60% in oil, 2.73 mmol) and $(BOC)_2O$ (0.34 g, 1.57 mmol) were reacted according to general procedure I, to give N-*tert*-butoxycarbonyl-N-benzyl-N'-cyclopropylmethylthiourea (0.26 g, 0.63 mmol, 46%); R_f =

0.60 [EtOAc/hexane (1:4)]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.00-0.04 [m, 2H, $NCH_2CH(CHHCHH)$], 0.13-0.19 [m, 2H, $NCH_2CH(CHHCHH)$], 0.26-0.32 [m, 1H, $NCH_2CH(CH_2CH_2)$], 1.21 [s, 9H, $C(CH_3)_3$], 3.20-3.26 (m, 2H, NCH_2), 3.99-4.08 (m, 1H, NCHH), 4.54-4.57 (m, 1H, NCHH) and 6.88-7.08 (m, 5H, ArH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 4.1 [$NCH_2CH(CH_2CH_2)$], 4.2 [$NCH_2CH(CH_2CH_2)$], 10.0 [$NCH_2CH(CH_2CH_2)$], 28.2 [$C(CH_3)_3$], 52.6 (NCH_2), 53.0 (NCH_2), 84.7 [$C(CH_3)_3$], 126.7 [C(2) and C(6)], 126.9 [C(4)], 128.4 [C(3) and C(5)], 138.9 [quaternary C(1)], 155.2 (C=O), 155.5 (C=O) and 183.6 (C=S); FAB$^+$MS *m/z* 321 [$(M+1)^+$, 55%] and 265 (100); HRMS (FAB) *m/z* 321.1639 $(M+1)^+$, $C_{17}H_{25}N_2O_2S$ requires 321.1637.

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N'-benzyl,N''-cyclopropylmethyl-thiourea)guanidinyl-3,14-di-hydroxyindolo[2',3':6,7]morphinan (117)

N-BOC-N-benzyl,N'-cyclopropylmethylthiourea (0.135 g, 0.32 mmol), 5'-amino-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-dihydroxyindolo[2',3':6,7]-morphinan (**120**) (0.100 g, 0.23 mmol), $HgCl_2$ (0.09 g, 0.32 mmol) and triethylamine (0.024 g, 0.033 ml, 0.23 mmol) were reacted according to general procedure F and stirred at 50 °C for 48 h, to give 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-BOC-(N'-benzyl,N''-cyclopropylmethyl)-guanidinyl-3,14-dihydroxyindolo[2',3':6,7]morphinan (0.058 g, 0.08 mmol, 35%); Rf = 0.47 [$CH_3OH/CH_2Cl_2/NH_4OH$ (89:10:1)]; ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.06-0.21 [m, 2 x 2H, $NCH_2CH(CHHCHH)$], 0.33-0.47 [m, 2H, $NCH_2CH(CHHCHH)$], 0.49-0.60 [m, 2H, $NCH_2CH(CHHCHH)$], 0.86-0.98 [m, 2 x 1H, $NCH_2CH(CH_2CH_2)$], 1.49 [s, 9H, $C(CH_3)_3$], 5.54 [s, 1H, C(5)H], 6.47-6.56 [m, 2H, C(1)H and C(2)H], 6.68-6.76 (m, 1H, ArH), 6.80-6.93 (m, 1H, ArH) and 7.01-7.48 (m, 6H, ArH); ^{13}C NMR (67.8 MHz, CD_3OD) δ 4.2 [$NCH_2CH(CH_2CH_2)$], 4.5 [$NCH_2CH(CH_2CH_2)$], 4.9 [$NCH_2CH(CH_2CH_2)$], 10.6 [$NCH_2CH(CH_2CH_2)$], 11.4 [$NCH_2CH(CH_2CH_2)$], 24.4 [C(10)], 28.9 [$C(CH_3)_3$], 29.0 (2 x CH_2), 29.1 (CH_2), 30.4 (CH_2), 33.0 (CH_2), 45.2 [quaternary C(13)], 60.8 [C(18)], 64.0 [C(9)], 74.7 [quaternary C(14)], 83.0 [quaternary $C(CH_3)_3$], 86.2 [C(5)], 111.2 (Ar), 112.3 (Ar), 119.1 (Ar), 120.0 (Ar), 125.4 (Ar), 128.3 (Ar), 128.9 (Ar), 129.0 (Ar), 129.5 (2 x Ar), 129.6 (2 x Ar), 130.0 (Ar), 132.0 (Ar), 132.3 (Ar), 136.1 (Ar), 138.6 (Ar), 142.8 (Ar), 145.4 (Ar), 155.2 (Ar) and 155.9 (C=N); FAB$^+$MS *m/z*

716 [$(M+1)^+$, 100%] and 616 (25); HRMS (FAB) *m/z* 716.3802 $(M+1)^+$, $C_{43}H_{50}N_5O_5$ requires 716.3812.

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-BOC-(N'-benzyl,N''-cyclopropylmethyl)-guanidinyl-3,14-dihydroxyindolo[2',3':6,7]morphinan (0.058 g, 0.08 mmol) was treated as described in general procedure G, to give 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N'-benzyl,N''-cyclopropylmethyl)guanidinyl-3,14-dihydroxyindolo[2',3':6,7]morphinan (**117**) as the bistrifluoroacetic acid salt (0.048 g, 0.08 mmol, 96%); mp. 157-158 °C; Rf = 0.08 [$CH_3OH/CH_2Cl_2/NH_4OH$ (89:10:1)]; IR ν_{max}/cm (KBr) 3800-2450 (br, bonded OH and NH) and 1679 (br, C=N, NH and NH_2); ^1H NMR (400 MHz, CD_3OD) δ 0.00-0.08 [m, 2H, $NCH_2CH(CHHCHH)$], 0.31-0.37 [m, 2H, $NCH_2CH(CHHCHH)$], 0.38-0.46 [m, 2H, $NCH_2CH(CHHCHH)$], 0.58-0.76 [m, 2H, $NCH_2CH(CHHCHH)$], 0.83-0.93 [m, 1H, $NCH_2CH(CH_2CH_2)$], 0.96-1.07 [m, 1H, $NCH_2CH(CH_2CH_2)$], 5.57 [s, 1H, C(5)*H*], 6.47-6.55 [m, 2H, C(1)*H* and C(2)*H*], 6.79-6.85 [m, 1H, Ar*H*], 7.09-7.23 [m, 1H, Ar*H*] and 7.27-7.32 [m, 1H, Ar*H*]; ^{13}C NMR (67.8 MHz, CD_3OD) δ 3.7 [$NCH_2CH(CH_2CH_2)$], 4.2 [$NCH_2CH(CH_2CH_2)$], 6.5 [$NCH_2CH(CH_2CH_2)$], 7.1 [$NCH_2CH(CH_2CH_2)$], 11.5 [$NCH_2CH(CH_2CH_2)$], 11.7 (CH_3), 23.6 [*C*(10)], 25.3 (CH_2), 30.0 (CH_2), 30.5 (CH_2), 44.6 [*C*(16)], 47.6 (CH_2), 47.9 [quaternary *C*(13)], 59.2 [*C*(18)], 63.9 [*C*(9)], 73.9 [quaternary *C*(14)], 85.2 [*C*(5)], 110.3 (Ar), 114.2 (Ar), 118.5 (Ar), 119.7 (Ar), 120.9 (Ar), 122.7 (Ar), 122.9 (Ar), 127.4 (Ar), 128.4 (2 x Ar), 128.9 (Ar), 129.2 (Ar), 130.1 (2 x Ar), 130.6 (Ar), 132.8 (Ar), 138.3 (Ar), 138.5 (Ar), 142.4 (Ar), 145.1 (Ar) and 156.8 (C=N); FAB^+MS *m/z* 616 [$(M+1)^+$, 100%]; HRMS (FAB) *m/z* 616.3276 $(M+1)^+$, $C_{38}H_{42}N_5O_3$ requires 616.3288.

17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N'-4-isothiocyanatobenzyl)-guanidinylethyl-3,14-dihydroxyindolo-[2',3':6,7]morphinan (124)

$NaHCO_3$ (0.013 g, 0.15 mmol) was added to 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-bis-BOC-(N'-4-aminobenzyl)guanidinylethyl-3,14-dihydroxyindolo-[2',3':6,7]morphinan (**126**) (0.030 g, 0.04 mmol) in a mixture of $CHCl_3$ (2 ml) and H_2O (1 ml). After cooling the reaction mixture in an ice bath, thiophosgene (0.006 g, 0.05 mmol) was added. The mixture was then

warmed to RT and allowed to stir for 2 h. The organic layer was collected and the aqueous layer further extracted with $CHCl_3$. The combined organic layers were then dried (Na_2SO_4), concentrated and purified by preparative thin layer chromatography [CH_3OH/CH_2Cl_2 (70:30)], to give 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-bis-BOC-(N'-4-isothiocyanatobenzyl)-guanidinylethyl-3,14-dihydroxyindolo-[2',3':6,7]morphinan (0.018 g, 0.02 mmol, 57%); Rf = 0.43 [CH_3OH/CH_2Cl_2 (70:30)]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.29-0.44 [m, 2H, $NCH_2CH(CHHCHH)$], 0.58-0.73 [m, 2H, $NCH_2CH(CHHCHH)$], 0.96-1.04 [m, 1H, $NCH_2CH(CH_2CH_2)$], 1.29 [s, 9H, $C(CH_3)_3$], 1.38 [s, 9H, $C(CH_3)_3$], 5.63 [s, 1H, C(5)*H*], 6.50 [d, J=8.2 Hz, 1H, C(1)*H*], 6.57 [d, J=8.2 Hz, 1H, C(2)*H*], 6.73-6.81 (m, 1H, Ar*H*) and 6.98-7.22 (m, 6H, Ar*H*); ^{13}C NMR adequate spectra were not obtained due to poor solubility. We aim to remake these compounds and to run ^{13}C NMR spectra in d^6-DMSO; FAB$^+$MS *m/z* 847 [$(M+1)^+$, 100%] and 647 (100); HRMS (FAB) *m/z* 847.3877 $(M+1)^+$, $C_{47}H_{55}N_6O_7S$ requires 847.3853.

17-Cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-bis-BOC-(N'-4-isocyanatobenzyl)guanidinyl-ethyl-3,14-dihydroxyindolo[2',3':6,7]morphinan (0.017 g, 0.02 mmol) was treated as described in general procedure G, to give 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N'-4-isocyanatobenzyl)guanidinylethyl-3,14-dihydroxyindolo[2',3':6,7]morphinan (**124**) as the bistrifluoroacetic acid salt (0.015 g, 0.02 mmol, 99%); mp. 154 °C; Rf = 0.16 [CH_3OH/CH_2Cl_2 (30:70)]; IR ν_{max}/cm (KBr) 3650-2850 (br, bonded OH and NH) and 1678 (br, C=N, NH and NH_2); ^{1}H NMR (400 MHz, CD_3OD) δ 0.48-0.59 [m, 2H, $NCH_2CH(CHHCHH)$], 0.73-0.92 [m, 2H, $NCH_2CH(CHHCHH)$], 1.10-1.21 [m, 1H, $NCH_2CH(CH_2CH_2)$], 5.75 [s, 1H, C(5)*H*], 6.64 [d, J=8.2 Hz, 1H, C(1)*H*], 6.67 [d, J=8.2 Hz, 1H, C(2)*H*], 6.84-6.90 (m, 1H, Ar*H*) and 6.99-7.32 (m, 6H, Ar*H*); ^{13}C adequate spectra were not obtained due to poor solubility. We aim to remake these compounds and to run ^{13}C NMR spectra in d^6-DMSO; FAB$^+$MS *m/z* 647 [$(M+1)^+$, 100%]; HRMS (FAB) *m/z* 647.2813 $(M+1)^+$, $C_{37}H_{39}N_6O_3S$ requires 647.2804.

17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-(N'-4-isothiocyanatobenzyl)-guanidinyl-3,14-dihydroxyindolo-[2',3':6,7]morphinan (125)

$NaHCO_3$ (0.020 g, 0.25 mmol) was added to 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-bis-BOC-(N'-4-aminobenzyl)guanidinyl-3,14-dihydroxyindolo-[2',3':6,7]morphinan (**127**) (0.048 g, 0.06 mmol) in a mixture of $CHCl_3$ (3 ml) and H_2O (1 ml). After cooling the reaction mixture in an ice bath, thiophosgene (0.009 g, 0.08 mmol) was added. The mixture was then warmed to RT and allowed to stir for 2 h. The organic layer was collected and the aqueous layer further extracted with $CHCl_3$. The combined organic layers were then dried (Na_2SO_4), concentrated and purified by preparative thin layer chromatography [CH_3OH/CH_2Cl_2 (70:30)], to give 17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-5'-bis-BOC-(N'-4-isothiocyanatobenzyl)-guanidinyl-3,14-dihydroxyindolo-[2',3':6,7]morphinan (**125**) (0.025 g, 0.03 mmol, 50%); Rf = 0.40 [CH_3OH/CH_2Cl_2 (70:30)]; ^1H NMR (400 MHz, $CDCl_3$) δ 0.08-0.17 [m, 2H, $NCH_2CH(CHHCHH)$], 0.48-0.57 [m, 2H, $NCH_2CH(CHHCHH)$], 1.36 [s, 9H, $C(CH_3)_3$], 1.38 [s, 9H, $C(CH_3)_3$], 5.67 [s, 1H, C(5)*H*], 6.60-6.78 (m, 3H, Ar*H*) and 7.03-7.54 (m, 6H, Ar*H*); ^{13}C NMR adequate spectra were not obtained due to poor solubility. We aim to remake these compounds and to run ^{13}C NMR spectra in d^6-DMSO; FAB^+MS *m/z* 819 [$(M+1)^+$, 100%] and 719 (20).

5.4 MOLECULAR MODELLING METHODS

This section contains a detailed description of the parameters used for the molecular modelling studies described in this thesis. The computational work was carried out under the guidance of Dr. Wolfgang Brandt, Martin-Luther Universiteit, Halle-Wittenberg, Halle (Saale), Germany.

5.4.1 Minimisation

The parameters used for the minimisation procedure were as follows:

Method: Powell	Initial Optimisation: simplex
Termination: Gradient [0.05 kcal/(mol*A)]	
Force Field: Tripos	Charges: Gasteiger-Marsili
Non-bonded cutoff: 8.0	
Dielectric Function: distance	Dielectric constant: 4.0
RMS Displacement: 0.001	
Min Energy Change: 0.05	Max Displacement: 0.01

At certain stages during the minimisation process, aggregates (atoms held fixed in their position) or constraints (distances between atoms maintained) were imposed on certain atoms. These were however removed before the final minimisation of the structures. One exception to this is the receptor. Whenever the receptor was minimised, either the backbone or the side chains were kept as aggregate. This was to ensure that the integrity of the receptor model was not jeopardised.

5.4.2 Molecular Dynamics

The molecule was heated to 100 K (Kelvin) for 10 000 fs (femtoseconds). The "dynamics history" was then replayed. A table was automatically generated, showing snap shots of the molecule at 1000 fs intervals. The molecule usually takes between 1000-2000 fs to equilibrate.

The latter snap shots and final structure of the molecule can therefore be minimised (according to the parameters discussed above) in order to find the minimum structure. By heating the molecule, it is hoped that the energy of the molecule would not rest in a local minimum, but rather that energy barriers might be overcome and that the molecule might finally rest in a global minimum state.

5.4.3 Simulated Annealing

The molecules were heated to 700 K (Kelvin) in 2000 fs (femtoseconds) and then cooled to 100 K in 2000 fs. This procedure was repeated 30 times. Analysis of the simulated annealing was run, generating a table with all the possible structures. The 30 structures with the lowest energy were automatically stored in a database (*ie.* lowest one from each run). These structures were however not minimised. Subsequent minimisation (according to the above procedure) allowed the structure with the approximate global minimum to be identified.

5.5 PHARMACOLOGICAL METHODS

This section contains a detailed description of the pharmacological procedures used to evaluate the compounds whose synthesis is described in this thesis. The displacement binding assays and functional *in vitro* assays (GPI, MVD, GTPγS) were performed under the National Institute on Drug Abuse (NIDA) contract, at Stanford Research Institute, California, USA.

5.5.1 *In Vitro* Assay Methods

Binding

Receptor binding studies were conducted on human opioid receptors transfected into Chinese hamster ovary (CHO) cells. The μ cell line is maintained in Ham's F-12 medium supplemented with 10% fetal bovine serum and 400μg/ml GENETICIN (G418 sulphate). The δ cell line is maintained in Ham's F-12 medium supplemented with 10% fetal bovine serum and 500μg/ml

hygromycin B. The κ cell line is maintained in Dulbecco's minimal essential medium (DMEM) supplemented with 10% fetal bovine serum, 400μg/ml GENETICIN (G418 sulphate) and 0.1% penicillin/streptomycin. All cell lines are grown to full confluency, then harvested for membrane preparation. The membrane used for functional assays is prepared in buffer A (20mM HEPES, 10mM $MgCl_2$, and 100mM NaCl at pH 7.4) and the membrane for binding assays is prepared in 50mM Tris buffer pH 7.7. Cells are harvested by scraping the plates with a rubber policeman and then centrifuged at 20000 x g for 20 minutes. The cell pellet is washed in buffer A or Tris, centrifuged at 20000 x g for another 20 minutes and finally suspended in a small amount of buffer to determine protein content. Membrane is aliquoted in small vials at a concentration of 6mg/ml per vial and stored at -70°C and used as needed.

Routine binding assays were conducted using [^{3}H] DAMGO, [^{3}H] Cl-DPDPE and [^{3}H] U69593 to bind to μ, δ and κ receptors, respectively. For binding, cell membranes are incubated with the appropriate radioligand and unlabelled drug in a total volume of 200μl in 96-well plates, usually for 1 hour at 25°C. For routine experiments, membranes are incubated with the test compounds at concentrations ranging from 10^{-5} to 10^{-10}M. After the incubation, samples are filtered through glass fibre filters by using a Tomtec cell harvester. Filters are dried overnight before radioactivity levels are determined. Non-specific binding is determined by using 1.0μM of the unlabelled countepart of each radioligand.

Full characterization of compounds includes analysis of the data for IC_{50} values and Hill coefficients by using the program PRISM. K_i values are calculated using the Cheng Prusoff transformation:

$$Ki = IC_{50}/(1+L/K_d)$$

where L is the radioligand concentration and K_d is the binding affinity of the radioligand, as determined previously by saturation analysis.

Functional - [^{35}S] GTPγS Assay

Membrane prepared as desribed above is incubated with [^{35}S] GTPγS (50pM), GDP (usually 10μM), and the desired compound, in a total volume of 200μl, for 60 minutes at 25°C. Samples are filtered over glass fibre filters and counted as described for the binding assays. A dose response curve with a prototypical full agonist (DAMGO, DPDPE, or U69593, for μ, δ and κ receptors, respectively) is conducted in each experiment to identify full and partial agonist compounds.

High affinity compounds that demonstrate no agonist activity are tested as antagonists. For each compound a full Schild analysis is conducted, utilizing a full agonist dose response curve in the presence of at least three concentrations of the antagonist. pA_2 values and Schild slopes are determined using a statistical program designed for these experiments. The equilibrium dissociation constant (K_e) is calculated from the following equation:

$$Ke = a / DR - 1$$

where "a" is the nanomolar concentration of antagonist and DR is the virtual shift of the agonist concentration-response curve to the right in the presence of a given concentration of antagonist.

6. REFERENCES

1. A.F. Casy and R.T. Parfitt, *Opioid Analgesics*, Plenum Press, London, 1986.

2. J.M. Gulland and R. Robinson, *J. Chem. Soc.*, 1923, 980.

3. M. Gates and G. Tschudi, J. Am. Chem. Soc., 1952, **74**, 1109.

4. W.R. Martin, *Pharmacol. Rev.*, 1967, **19**, 463.

5. J.A.H. Lord, A.A. Waterfield, J. Hughes and H.W. Kosterlitz, *Nature*, 1977, **267**, 495; W.R. Martin, C.G. Eades, J.A. Thompson, R.E. Huppler and P.E. Gilbert, *J. Pharmac. Exp. Ther.*, 1976, **197**, 517; P.E. Gilbert and W.R. Martin, *J. Pharmac. Exp. Ther.*, 1976, **198**, 66.

6. C.J. Evans, D.E. Keith, H. Morrison, K. Magendzo and R.H. Edwards, *Science*, 1992, **258**, 1952; B.L. Kieffer, K. Befort, C. Gaveriaux-Ruff and C.G. Hirth, *Proc. Natl. Acad. Sci. USA*, 1992, **89**, 12048; Y. Chen, A. Mestek, J. Liu, J.A. Hurley and L. Yu, *Mol. Phamacol.*, 1993, **44**, 8.

7. T.G. Metzger and D.M. Ferguson, *FEBS Lett.*, 1995, **375**, 1.

8. J. Hughes, T.W. Smith, B.A. Morgan and L.A. Fothergill, *Life Science*, 1975, **16**, 1753.

9. J. Hughes, T.W. Smith, H.W. Kosterlitz, L.A. Fothergill, B.A. Morgan and H.R. Morris, *Nature*, 1975, **258**, 577.

10. J.D. Belluzzi, N. Grant, V. Garsky, D. Sarantakis, C.D. Wise and L. Stein, *Nature*, 1976, **260**, 625.

11. L.J. Traficante, J. Rotrosen, J. Siekierski, H. Tracer and S. Gershon, *Life Science*, 1980, **26**, 1697; B. Malfray, J.P. Swerts, A. Guyon, B.P. Roques and J.C. Swartz, *Nature*, 1978, **276**, 523.

12. H.H. Loh, L.F. TSeng, E. Wei, C.H. Li, *Proc. Natl. Acad. Sci. USA*, 1976, **73**, 2895.

13. C.B. Pert, A. Pert, J.K. Chang and B.T.W. Fong, *Science*, 1976, **194**, 330; J.S. Morley, *Ann. Rev. Pharmacol. Toxicol.*, 1980, **20**, 81.

14. C.H. Li, L. Barnafi, M. Chretien and D. Chung, *Nature*, 1965, **208**, 1093.

15. A.F. Bradbury, D.G. Smyth, C.R. Snell, N.J.M. Birdsall and E.C. Hulme, *Nature*, 1976, **260**, 793.

16. B.M. Cox, K.E. Opheim, H. Teschemacher and A. Goldstein, *Life Science*, 1975, **16**, 1777.

17. H. Schmidhammer, *Prog. Med. Chem.*, 1998, **35**, 83.

18. J.W. Lewis, K.W. Bentley and A. Cowan, *Ann. Rev. Pharmacol. Toxicol.*, 1971, **11**, 241.

19. H.W. Kosterlitz, H.O.J. Collier and J.E. Villarreal, eds., *Agonist and Antagonist Actions of Narcotic Analgesic Drugs*, University Park Press, Baltimore, 1973.

20. J.D. Wallach, *Vet. Med.*, 1961, **64**, 53.

21. S.N. Downing, W.P. Leary and E.S. White, *Br. J. Anaesth.*, 1977, **47**, 251.

22. P.A.J. Janssen, *Br. J. Anaesthesia*, 1962, **34**, 260.

23. J.B. Thomas, M.J. Fall, J.B. Cooper, R.B. Rothman, S.W. Mascarella, H. Xu, J.S. Partilla, C.M. Dersch, K.B. McCullough, B.E. Cantrell, D.M. Zimmerman and F.I. Carroll, *J. Med. Chem.*, 1998, **41**, 5188.

24. G. Dandio, S. Ronzini and P. Petrillo, *Exp. Opin. Ther. Patents*, 1997, **7**, 1075.

25. J.K. Chang, G.C. Rigdon, J.L. Howard and R.W. McNutt, *J. Pharmacol. Exp. Ther.*, 1993, **267**, 852.

26. E.J. Bilsky, S.N. Calderon, T. Wang, R.N. Bernstein, P. Davis, V.J. Hruby, R.W. McNutt, R.B. Rothman, K.C. Rice and F. Porreca, *J. Pharmacol. Exp. Ther.*, 1995, **273**, 359.

27. R.A. Lahti, P.F. Von Voightlander and C. Barsuhn, *Life Sci.* 1982, **31**, 2257.

28. A. Samhammer, U. Holzgrabe and R. Haller, *Arch. Pharm. (Weinheim)*, 1989, **322**, 551.

29. G. Ronsisvalle, O. Prezzavento, L. Pasquinucci, A. Marrazzo, F. Vittorio, J.A. Gomez-Vidal, L. Carboni and S. Spampinato, *Life Sci.*, 1995, **57**, 1487.

30. K. Unna, *J. Pharmacol. Exp. Ther.*, 1943, **79**, 27; J. Weijlad and A.E. Erickson, *J. Am. Chem. Soc.*, 1942, **64**, 869.

31. L. Lasagna and H.K. Beecher, *J. Pharmacol. Exp. Ther.*, 1954, **112**, 356; A.S. Keats and J. Telford, *J. Pharmacol. Exp. Ther.*, 1956, **117**, 190.

32. J.W. Holaday and A.J. Faden, *Nature*, 1978, **275**, 450; A.J. Faden and J.W. Holaday, *J. Pharmacol. Exp. Ther.*, 1980, **212**, 441.

33. W.R. Martin, D.R. Jasinski and P.A. Mansky, *Arch. Gen. Psychiatr.*, 1973, **28**, 784.

34. R.C. Pitts, R.M. Allen, E.A. Walker and C.A. Dykstra, *J. Pharmacol. Exp. Ther.*, 1998, **285**, 1197.

35. H. Schmidhammer, W.P. Burkard, L. Eggstein-Aeppli and C.F.C. Smith, *J. Med. Chem.*, 1989, **32**, 418.

36. D.M. Zimmerman, R. Nickander, J.S. Horng and D.T. Wong, *Nature*, 1978, **275**, 332.

37. J.B. Thomas, S.W. Mascarella, R.B. Rothman, J.S. Partilla, H. Xu, K.B. McCullough, C.M. Dersch, B.E. Cantrell, D.M. Zimmerman and F.I. Carroll, *J. Med. Chem.*, 1998, **41**, 1980.

38. P.S. Portoghese, D.L. Larson, J.B. Jiang, A.E. Takemori and T.P. Caruso, *J. Med. Chem.*, 1978, **21**, 598.

39. P.S. Portoghese, D.L. Larson, L.M. Sayre, D.S. Fries and A.E. Takemori, *J. Med. Chem.*, 1980, **23**, 233.

40. S.J. Ward, P.S. Portoghese and A.E. Takemori, *J. Pharmacol. Exp. Ther.*, 1982, **220**, 494; S.J. Ward, P.S. Portoghese and A.E. Takemori, *Eur. J. Pharmacol.*, 1982, **80**, 377.

41. J.W. Lewis, C.F.C. Smith, P.S. McCarthy, D.S. Walter, R.J. Kobylecki, M. Myers, A.S. Haynes, C.J. Lewis and K. Waltham, *NIDA Res. Monog.*, 1989, **90**, 136.

42. S.D. Comer, T.F. Burke, J.W. Lewis and J.H. Woods, *J. Pharmacol. Exp. Ther.*, 1992, **262**, 1051.

43. N. Nieland, Ph.D. Thesis, University of Bristol, 1998.

44. A. Sebastian, J.M. Bidlack, Q. Jiang, D. Deecker, M. Teitler, S.D. Glick and S. Archer, *J. Med. Chem.*, 1993, **36**, 3154.

45. J.H. Broadbear, T.L. Sumpter, T.F. Burke, S.M. Husbands, J.W. Lewis, J.H. Woods and J.R. Traynor, *J. Pharmacol. Exp. Ther.*, 2000, **294**, 933.

46. I. Derrick, C.L. Neilan, J. Anders, S.M. Husbands, J.H. Woods, J.R. Traynor and J.W. Lewis, *J. Med. Chem.*, 2000, **43**, 3348.

47. P.W. Schiller, *Prog. Med. Chem.*, 1991, **28**, 301.

48. P.W. Schiller, T.M.-D. Nguyen, G. Weltrowska, B.C. Wilkes, B.J. Marsden, C. Lemieux and N.N. Chung, *Proc. Natl. Acad. Sci. USA*, 1992, **89**, 11871.

49. S. Salvadori, M. Attila, G. Balboni, C. Bianchi, S.D. Bryant, O. Crescenzi, R. Guerrini, D. Picane, T. Tancredi, P.A. Temussi and L.H. Lazarus, *Mol. Med.*, 1995, **1**, 678.

50. P.S. Portoghese, M. Sultana, H. Nagase and A.E. Takemori, *J. Med. Chem.,* 1988, **31**, 281.

51. P.S. Portoghese, H. Nagase and A.E. Takemori, *J. Med. Chem.*, 1988, **31**, 1344.

52. P.S. Portoghese, *J. Med. Chem.*, 1992, **35**, 1927.

53. K. Arakawa, T. Akami, M. Okamoto, T. Oka, H. Nagase and S. Matsumoto, *Transplantation*, 1992, **53**, 951; K. Arakawa, T. Akami, M. Okamoto, H. Nakajima, M. Mitsuo, I. Naka, T. Oka and H. Nagase, *Transplant. Proc.*, 1992, **24**, 696.

54. H. Nagase, A. Mizusuna, K. Kawai and I. Nakatani, *Chem. Abstr.*, 1994, **121**, P134540b; P.S. Portoghese, M. Sultana and A.E. Takemori, *J. Med. Chem.*, 1990, **33**, 1714; S. Ananthan, C.A. Johnson, R.L. Carter, S.D. Clayton, K.C. Rice, H. Xu, P. Davis, F. Porreca and R.B. Rothman, *J. Med. Chem.*, 1998, **41**, 2872.

55. G. Dondio, G.D. Clarke and G. Giardina, *Analgesia*, 1995, 1, 394; *Chem. Abstr.*, 1992, **116**, p255598g; *Chem. Abstr.*, 1995, **122**, p314536j.

56. K.C. Rice, A.E. Jacobson, T.R. Burke, Jr., B.S. Bajwa, R.A. Streaty and W.A. Klee, *Science*, 1983, **220**, 314.

57. T.R. Burke, Jr., A.E. Jacobson, K.C. Rice, J.V. Silverton, W.F. Simmonds, R.A. Streaty

and W.A. Klee, *J. Med. Chem.*, 1986, **29**, 1087.

58. P.S. Portoghese, M. Sultana and A.E. Takemori, *J. Med. Chem.*, 1990, **33**, 1547.

59. V.L. Korlipara, A.E. Takemori and P.S. Portoghese, *J. Med. Chem.*, 1995, **38**, 1337.

60. V.L. Korlipara, A.E. Takemori and P.S. Portoghese, *J. Med. Chem.*, 1994, **37**, 1882;

61. P.W. Schiller, Y. Lu, G. Weltrowska, I. Berezowska, B.C. Wilkes and T.M.-D. Nguyen, 32nd International Narcotics Research Conference, Helsinki, 2001.

62. P.S. Portoghese, A.W. Lipkowski and A.E. Takemori, *Life Science*, 1987, **40**, 1287; A.E. Takemori, B.Y. Ho, J.S. Naeseth and P.S. Portoghese, *J. Pharmacol. Exp. Ther.*, 1988, **246**, 255.

63. D.M. Zimmerman and J.D. Leander, *J. Med. Chem.*, 1990, 33, 895.

64. D.C. Jewett anad J.H. Woods, *Behav. Pharm.*, 1995, **6**, 815.

65. C.A. Paronis, A.B. Waddell and S.G. Holtzman, *Pharmacology Biochemistry and Behaviour*, 1994, 46, 813.

66. C.-E. Lin, A.E. Takemori and P.S. Portoghese, *J. Med. Chem.*, 1993, **36**, 2412.

67. S.L. Olmsted, A.E. Takemori and P.S. Portoghese, *J. Med. Chem.*, 1993, **36**, 179.

68. Xue, J.-C., Chen, C., Zhu, J., Kunapuli, S., DeRiel, J.K., Yu, L. and Liu-Chen L.-Y., *J. Bio. Chem.*, 1994, **269**, 30195.

69. Hjorth, S.A., Thirstrup, K., Grandy, D.K. and Schwartz, T.W., *Mol. Pharmacol.*, 1995, **47**, 1089.

70. T. Seki, M. Minami, T. Nakagawa, Y. Ienaga, A. Morisada and M. Satoh, *Eur. J. Pharmacol.*, 1998, **350**, 301.

71. D.L. Larson, R.M. Jones, S.A. Hjorth, T.W. Schwartz and P.S. Portoghese, *J. Med. Chem.*, 2000, **43**, 1573.

72. P.S. Portoghese and S.L. Olmsted, US Patent, 1995, 5 457 208.

73. A.R. Jales, S.M. Husbands and J.W. Lewis, *Bioorg. Med. Chem. Lett.*, 2000, **10**, 2259.

74. A.R. Jales, Ph.D. Thesis, University of Bristol, 1999.

75. R.M. Jones, S.A. Hjorth, T.W. Schwartz and P.S. Portoghese, *J. Med. Chem.*, 1998, **41**, 4911.

76. S.K. Sharma, R.M. Jones, T.G. Metzger, D.M. Ferguson and P.S. Portoghese, *J. Med. Chem.*, 2001, **44**, 2073.

77. W.C. Stevens, Jr., R.M. Jones, G. Subramanian, T.G. Metzger, D.M. Ferguson and P.S. Portoghese, *J. Med. Chem.*, 2000, **43**, 2759.

78. S.S. Negus, N.K. Mell, R.M. Jones and P.S. Portoghese, *College on Problems of Drug Dependence*, Scottsdale, Arizona, 2001.

79. J.B. Thomas, R.N. Atkinson, R.B. Rothman, S.E. Fix, S.W. Mascarella, N.A. Vinson, H. Xu, C.M. Dersch, Y.-F. Lu, B.E. Cantrell, D.M. Zimmerman and F.I. Carroll, *J. Med. Chem.*, 2001, **44**, 2682.

80. B.R. De Costa, L. Brand, R.B. Rothman, A.E. Jacobsen, V. Bykov and K.C. Rice, *J. Med. Chem.*, 1989, **32**, 281.

81. A. Chang, A.E. Takemori and P.S. Portoghese, *J. Med. Chem.*, 1994, **37**, 1547.

82. W.F. Michne and N.E. Albertson, *J. Med. Chem.*, 1972, **15**, 1278.

83. Y.L. Hurd and M. Herkenham, *Synapse*, 1993, **13**, 357; J.B. Daunais and J.F. McGinty, *Synapse*, 1994, **18**, 35; J.B. Daunais and J.F. McGinty, *Mol. Brain Res.*, 1995, **29**, 201.

84. C.A. Crawford, S.A. McDougall, C.A. Bolanos, S. Hall and S.P. Berger, *Psychopharmacol.*, 1995, **120**, 392; C.A. Heidbreder, D. Babovic-Vuksanovic, M. Shoaib and T.S. Shippenberg, *J. Pharmacol. Exp. Ther.*, 1995, **275**, 150; T.S. Shippenberg, A. LeFavour and C.A. Heidbreder, *J. Pharmacol. Exp. Ther.*, 1996, **276**, 545.

85. S.D. Glick, I.M. Maisonneuve, J. Raucci and S. Archer, *Brain Res.*, 1995, **681**, 147.

86. D. Woolfolk and S.G. Holtzman, *FASEB*, 1996, **10**, 2583.

87. R.B. Rothman, D.A. Gorelick, P.R. Eichmiller, B.H. Hill, J. Norbeck and J.G. Liberto, *NIDA Res. Monog.*, 1997, **178**, 309.

88. S.S. Negus, S.J. Henriksen, A. Mattox, G.W. Pasternak, P.S. Portoghese, A.E. Takemori, M.B. Weinger and G.F. Koob, *J. Pharmacol. Exp. Ther.*, 1993, **265**, 1245.

89. C. Gueniau and C. Oberlander, *J. Pharmacol. Exp. Ther.*, 1997, **282**, 1.

90. D.D. Newman, N. Rajakumar, B.A. Flumerfelt and A.J. Stoessl, *Neuroreport*, 1997, **8**, 669.

91. R. Vink, P.S. Portoghese and A.I. Faden, *Am. J. Physiol.*, 1991, **261**, R1527.

92. T. Katafuchi, Y. Hattori, I. Nagatomo and K. Koizumi, *Brain Res.*, 1991, **546**, 1.

93. A.L.J. Beckwith, *The Chemistry of Amides*, J. Zabicky ed., Wiley, New York, 1970, 73.

94. A.L.J. Beckwith, *The Chemistry of Amides*, J. Zabicky ed., Wiley, New York, 1970, 86; R Kluger and J.C. Hunt, *J. Am. Chem. Soc.*, 1989, **111**, 3325.

95. A.L.J. Beckwith, *The Chemistry of Amides*, J. Zabicky ed., Wiley, New York, 1970, 96.

96. Y.S. Klausner and M. Bodansky, *Synthesis*, 1972, 453.

97. K. Ishihara, S. Ohara and H. Yamamoto, *J. Org. Chem.*, 1996, **61**, 4196.

98. J. Klosa, *J. Prakt. Chem.*, 1963, **19**, 45.

99. B. Castro, J.R. Dormoy, G. Evin and C. Selve, *Tetrahedron Lett.*, 1975, **14**, 1219.

100. M.C. Desai and L.M. Stephens Stramiello, *Tetrahedron Lett.*, 1993, **34**, 7685; A. Hassner and V. Alexanian, *Tetrahedron Lett.*, 1978, 4475.

101. J.C. Grivas and A Taurins, *Can. J. Chem.*, 1961, **39**, 761.

102. P. Oxley, M.W. Partridge and W.F. Short, *J. Chem. Soc.*, 1947, 1110.

103. J.H. Forsberg, V.T. Spziano, T.M. Balasubramanian, G.K. Liu, S.A. Kinsley, C.A. Duckworth, J.J. Poeruca, P.S. Brown and J.L. Miller, *J. Org. Chem.*, 1987, **52**, 1017; R.S. Garigipati, *Tetrahedron Lett.*, 1990, **31**, 1969.

104. J.D. Wilson, J.S. Wager and H. Weingarten, *J. Org. Chem.*, 1971, **36**, 1613; A.B. Charette and M. Grenon, *Tetrahedron Lett.*, 2000, **41**, 1677.

105. G. Glock, *Berichte*, 1888, 2650.

106. P. Reynaud, R.C. Moreau and T.C. Gousson, *R. Acad. Sci.*, 1964, **259**, 4067.

107. M.J. Winchester and F.D. Popp, *J. Het. Chem.*, 1975, **83**, 547.

108. R. Baati, V. Gouverneur and C. Mioskowski, *Synthesis*, 1999, **6**, 927.

109. L.P. Kyrides, F.B. Zientry, G.W. Steahly and H.L. Morrill, *J. Org. Chem.*, 1947, **12**, 577.

110. P. Oxley and W.F. Short, *J. Chem. Soc.*, 1947, 497.

111. P.T. Charlton, G.K. Maliphant, P. Oxley and D.A. Peak, *J. Chem. Soc.*, 1951, 485.

112. E. Waldmann and A. Chwala, *Chem. Ber.*, 1941, **74**, 1763 and references therein.

113. S.R. Aspinall, *J. Am. Chem Soc.*, 1939, **61**, 3195.

114. R.N. Butler and K.J. Fitzgerald, *J. Chem Soc. Perkin Trans. I*, 1989, 155.

115. D.P.N Satchell and R.S. Satchell, *Chem. Soc. Rev.*, 1975, **4**, 231.

116. J. Anatol and J. Berecoechea, *Synthesis*, 1975, 111.

117. K.A. Hackl and H. Falk, *Monatsh Chem, EN*, 1992, **123**, 599.

118. M. Anbazhagan, A.R.A.S. Deshmukh and S. Rajappa, *Tetraheron Lett.*, 1998, **39**, 3609.

119. T.D. Nelson, R.D. Davis and W.L. Nelson, *J. Med. Chem.*, 1994, **37**, 4270.

120. A.Geal, Ph.D. Thesis, University of Bath, 1999.

121. T.P. Curran, M.P. Pollastri, S.M. Abelleira, R.J. Messier, T.A. McCollum and C.G. Rowe, *Tetrahedron Lett.*, 1994, **35**, 5409.

122. R.F. Borch, M.D. Bernstein and H.D. Durst, *J. Am. Chem. Soc.*, 1971, **93**, 2897

123. I. Saxena, R. Borah and J.C. Sarma, *J. Chem Soc., Perkin Trans. 1*, 2000, 503.

124. B.C. Challis and J.A. Challis, *The Chemistry of Amides*, J. Zabicky ed., Wiley, New York, 1970, 795.

125. R.L. Augustine, *Reduction*, Marcel Dekker, New York, 1968.

126. H.C. Brown, S. Narasimhan and Y.M. Choi, *Synth. Comm.*, 1981, 996.

127. A.S.B. Prasad, J.V.B. Kanth and M. Perisamy, *Tetrahedron*, 1992, **48**, 4623.

128. R.L. Larock, *Comprehensive Organic Transformations*, VCH, New York, 1989, 624.

129. E. Mosettig, *Organic Reactions*, 8, R. Adams, A.H. Blatt, A.C. Cope, D.Y. Curtin, F.C. McGrew and C. Niemann, eds.; Wiley, New York, 1954, 246.

130. A.E.G. Miller, J.W. Biss and L.H. Schwartzman, *J. Org. Chem.*, 1959, **24**, 627; J.A. Marshall, N.H. Andersen and J.W. Schlicher, *J. Org. Chem.*, 1970, **35**, 858.

131. T. van Es and B. Staskun, *Org. Synth. Coll. vol.*, 1988, **6**, 631; L. Katz and W. Schroeder, *J. Org. Chem.*, 1954, 19, 103; G. Knupp and A.W. Frahm, *J. Chem. Res. (S)*, 1981, 164.

132. J.W. Bae, S.H. Lee, Y.J. Cho and C.M. Yoon, *J. Chem. Soc., Perkin Trans. 1*, 2000, 145.

133. D.G. Lee, *Oxidation in Organic Chemistry*, Trahanovsky ed., Academic Press, New York, pt. D, 147.

134. A.M. Maione and A. Romeo, *Synthesis*, 1984, 955.

135. R.N. Warrener, T.S. Lee, R.A. Russell and M.N. Paddon-Row, *Aust. J. Chem.*, 1978, **31**, 1113.

136. M.E. Krafft and B. Zorc, *J. Org. Chem.*, 1986, **51**, 5482.

137. A.J. Mancuso and D. Swern, *Synthesis*, 1981, 165.

138. D.B. Dess and J.C. Martin, *J. Org. Chem.*, 1983, **48**, 4155.

139. A.J. Geall and I.S. Blagbrough, *Tetrahedron*, 2000, **56**, 2449.

140. C.A. Maryanoff, R.C. Stanzione, J.N. Plampin and J.E. Mills, *J. Org. Chem*, 1986, **51**, 1882 and references therein.

141. K. Kim, Y.-T. Lin and H.S. Mosher, *Tetrahedron Lett.*, 1988, **29**, 3183.

142. K. Feichtinger, C. Zapf, H.L. Sings and M. Goodman, *J. Org. Chem*, 1998, **63**, 3804 and references therein.

143. D.S. Dodd and A.P. Kozikowski, *Tetrahedron Lett.*, 1994, **35**, 977.

144. M.A. Poss, E. Iwanowicz, J.A. Reid, J. Lin and Z. Gu, *Tetrahedron Lett.*, 1992, **33**, 5933; K.S. Kim and L. Qian, *Tetrahedron Lett.*, 1993, **34**, 7677.

145. K.S. Atwal, S.Z. Ahmed and B.C. O'Reilly, *Tetrahedron Lett.*, 1989, **30**, 7313 and references therein.

146. C. Levallet, J. Lerpiniere and S.Y. Ko, *Tetrahedron*, 1997, **53**, 5291 and references therein.

147. S.Y. Ko, J. Lerpiniere and A.M. Christofi, *Synlett.*, 1995, 815.

148. I.D. Linney, I.M. Buck, E.A. Harper, S.B. Kalindjian, M.J. Pether, N.P. Shankley, G.F. Watt and P.T Wright, *J. Med. Chem.*, 2000, **43**, 2362.

149. D.R. Kent, W.L. Cody and A.M. Doherty, *Tetrahedron Lett.* 1996, **37**, 8711.

150. S.J. Angyal and W.K. Warburton, *J. Chem. Soc.*, 1951, 2492.

151. J.D. Mold, J.M. Ladino and E.J. Schantz, 1953, 6321.

152. E. Junod, *Helv. Chim. Acta*, 1952, **35**, 1005.

153. K. Ramadas and N. Srinivasan, *Tetrahedron Lett.*, 1995, **36**, 2841.

154. S.M. Husbands, S. Izenwasser, R.J. Loeloff, J.L. Katz, W.D. Bowen, B.J. Vilner and A. Hauck Newman, *J. Med. Chem.*, 1997, **40**, 4340.

155. Q. Jiang, A. Seyed-Mozaffari, S. Archer, and J.M. Bidlack, *J. Pharmacol. Exp. Ther.*, 1992, **262**, 526.

156 A. Thurkauf, M.V. Mattson, P.N. Huguenin, K.C. Rice and A.E. Jacobson, *FEBS Letts.*, 1988, **238**, 369.

157. R.L. Frank and P.V. Smith, *Organic Syntheses, Coll. Vol 3*, 735.

158. O. Tsuge, *The Chemistry of Cyanates and Their Thio Derivatives*, S. Patai ed., pt.1, Wiley, New York, 1977, 445.

159. J. March, *Advanced Organic Chemistry*, 4th Ed., Wiley, New York, 1992, 670.

160. S. Tanaka, S. Uemura and M. Okano, *Bull. Chem. Soc. Jpn.*, 1977, **50**, 2785.

161. P. Molina, M. Alajarin and A. Arques, *Synthesis*, 1982, 596.

162. M. Yokoyama and T. Imamoto, *Synthesis*, 1984, 797.

163. S. Ozaki, *Chem. Rev.*, 1972, **72**, 457.

164. J.H. Jaffe and W.R. Martin, *The Pharmacological Basis of Therapeutics*, A.G. Gilman, L.S. Goodman and A. Gilman (eds), 6th edn., MacMillan Publishing Co., Inc., New York, 1980, p 496; J.W. Lewis, *Buprenorphine*, A. Cowan and J.W. Lewis (eds), Wiley-Liss, Inc., New York, 1995, p 3.

165. W. Brandt, S. Drosihn, M. Haurand, U. Holzgrabe and C. Nachtsheim, *Arch. Pharm. Pharm. Med. Chem.*, 1996, **329**, 311.

166. C. Cometta-Morini and G.H. Loew, *J. Comput. -Aided Mol. Design*, 1991, **5**, 335; C. Cometta-Morini, P.A. Maguire and G.H. Loew, *Mol. Pharmacol.*,1992, **41**, 185.

167. A. Lavecchia, G. Greco, E. Novellino, F. Vittorio and G. Ronsisvalle, *J. Med. Chem.*, 2000, **43**, 2124.

168. M. Filizola, H.O. Villar and G.H. Loew, *Bioorg. Med. Chem.*, 2001, **9**, 69.

169. G. Subramanian, M.G. Paterlini, D.L. Larson, P.S. Portoghese and D.M. Ferguson, *J. Med. Chem.*, 1998, **41**, 4777 and references therein.

170. K. Palczewski, T. Kumasaka, T. Hori, C.A. Behnke, H. Motoshima, B.A. Fox, I. Le Trong, D.C. Teller, T. Okada, R.E. Stenkamp, M. Yamamoto and M. Miyano, *Science*, 2000, **289**, 739.

171. R. Henderson, J. Baldwin, T.H. Ceska, F. Zemlin, E. Beckmann and K. Downing, *J. Mol. Biol.*, 1990, **213**, 899.

172. S. Trumpp-Kallmeyer, J. Hoflack, A. Bruinvels and M. Hibert, *J. Med. Chem.*, 1992, **35**, 3448.

173. S.G. Dahl, O. Edvarsen and I. Sylte, *Proc. Natl Acad. Sci. USA*, 1991, **88**, 8111; J. Groetzinger, M. Engels, E. Jacoby, A. Wollmer and W. Strassburger, *Protein Eng.*, 1991, 4, 767; Y. Yamamoto, K. Kamiya and S. Terao, *J. Med. Chem.*, 1993, **36**, 820; G. Nordvall and U.J. Hacksell, *J. Med. Chem.*, 1993, **36**, 967; D. Roeper, P. Krueger, J. Groetzinger, A. Wollmer and W. Strassburger, *Receptors and Channels*, 1995, **3**, 97; F. Fanelli, M.C. Menziani, M. Cocchi and P.G. De Benedetti, *J. Mol. Struct.*, 1995, **333**, 49; A. Vedani, P. Zbinden, J.P. Snyder and P.A. Greenidge, *J. Am Chem. Soc.*, 1995, **117**, 4987.

174. G.F.X. Schertler, C. Villa and R. Henderson, *Nature*, 1993, **362**, 770.

175. I. Alkorta and G.H. Loew, *Protein Eng.*, 1996, **9**, 573; I. Alkorta and P. Du, *Protein Eng.*, 1994, **7**, 1231.

176. J.A. Ballesteros and H. Weinstein, *Meth. Neurosci.*, 1995, **25**, 366.

177. V.M. Unger, P.A. Hargrave, J.M. Baldwin and G.F.X. Schertler, *Nature*, 1997, **389**, 203.

178. H. Kong, K. Raynor and T. Reisine, *Regulatory Peptides*, 1994, **54**, 155.

179. T.G. Metzger, M.G. Paterlini, P.S. Portoghese and D.M. Ferguson, *Neurochem. Res.*, 1996, **21**, 1287.

180. P.S. Portoghese, H. Nagase, A.W. Lipkowski, D.L.. Larson and A.E. Takemori, *J. Med. Chem.*, 1988, **31**, 836.

181. G.R. Uhl, S. Childers and G. Pasternak, *TINS*, 1994, **17**, 89; C.K. Surratt. P.S. Johnson, A. Moriwaki, B.K. Seidleck, C.J. Blaschak, J.B. Wong and G.R. Uhl, *J. Biol. Chem.*, 1994, **269**, 20548.

182. M. Stahl and M. Rarey, *J. Med. Chem.*, 2001, **44**, 1035.

183. H.-J. Boehm, *J. Comput. –Aided. Mol. Design*, 1994, **8**, 243.

184. T.L. Blundell, D.P. Carney, S. Gardner, F.R.F. Hayes, B. Howlin, T.J.P. Hubbard, J.P. Overington, D.A. Singh, B.L. Sibanda and M.J. Sutcliffe, *Eur. J. Biochem.*, 1988, **172**, 513; M.J. Sutcliffe, I. Haneef, D.P. Carney and T.L. Blundell, *Prot. Eng.*, 1987, **1**, 377; M.J. Sutcliffe, F.R.F. Hayes and T.L. Blundell, *Prot. Eng.*, 1987, **1**, 385.

185. R.A. Laskowski, M.W. MacArthur, D.S. Moss and J.M. Thornton, *J. Appl. Cryst.*, 1993, **26**, 283.

186. H.-J. Boehm, *J. Comput. –Aided. Mol. Design*, 1992, **6**, 61; H.-J. Boehm, *J. Comput. – Aided. Mol. Design*, 1992, **6**, 593; G. Klebe, *J. Mol. Biol.*, 1994, **237**, 221.

187. SYBYL Interface to FlexX, Receptor-based Design Manual, Version 6.7, October 2000, Tripos Inc.

188. R. Wang, L. Liu, L. Lai and Y. Tang, *J. Mol. Model.*, 1998, **4**, 379.

189. Wolfgang Brandt, personal communication to S.L. Black

190. R. Schwyzer, *Biochemistry*, 1986, **25**, 6335.

191. R.I. Taber, *Adv. Biochem. Psychopharmacol.*, 1974, **8**, 191.

192. H.W. Kosterlitz and A.J. Watt, *Br. J. Pharmacol.*, 1968, **33**, 266; G. Henderson, J. Hughes and H.W. Kosterlitz, *Br. J. Pharmacol.*, 1972, **46**, 764.

193. F.M. Leslie, *Pharm. Rev.*, 1987, **39**, 197.

194. L. Toll, I.P. Berzetei-Gurske, W.E. Polgar, S.R. Brandt, I.D. Adapa, L.Rodriguez, R.W. Schwartz, D. Haggart, A. O'Brien, A. White, J.M. Kennedy, K. Craymer, L. Farrington and J.S. Aul, *NIDA Res. Monog.*, 1997, **178**, 440.

195. Y.C. Cheng and W.H. Prusoff, *Biochem. Pharmacol.*, 1973, **22**, 3099; T.C. Chou, *Mol. Pharmacol.*, 1974, **10**, 235.

196. W.D.M. Paton, *Br. J. Pharmacol. Chemother.*, 1957, **12**, 119.

197. J.R. Traynor and S.R. Nahorski, *Mol. Pharmacol.*, 1995, **47**, 848.

198. J.U. Adams, C.A. Paronis and S.G. Holtzman, *J. Pharmacol. Exp. Ther.*, 1990, **255**, 1027.

199. G. Woolfe and A.D. McDonald, *J. Pharmacol. Exp. Ther.,* 1944, **80**, 300.

200. A.G. Hayes, M.J. Sheehan and M.B. Tyers, *Br. J. Pharmacol.*, 1987, **91**, 832.

201. M.B. Tyers, *Br. J. Pharmacol.*, 1980, **69**, 503.

202. www.opioid.umn.edu

203. M.G. Paterlini, P.S. Portoghese and D.M. Ferguson, *J. Med. Chem.*, 1997, **40**, 3254.

7. APPENDICES

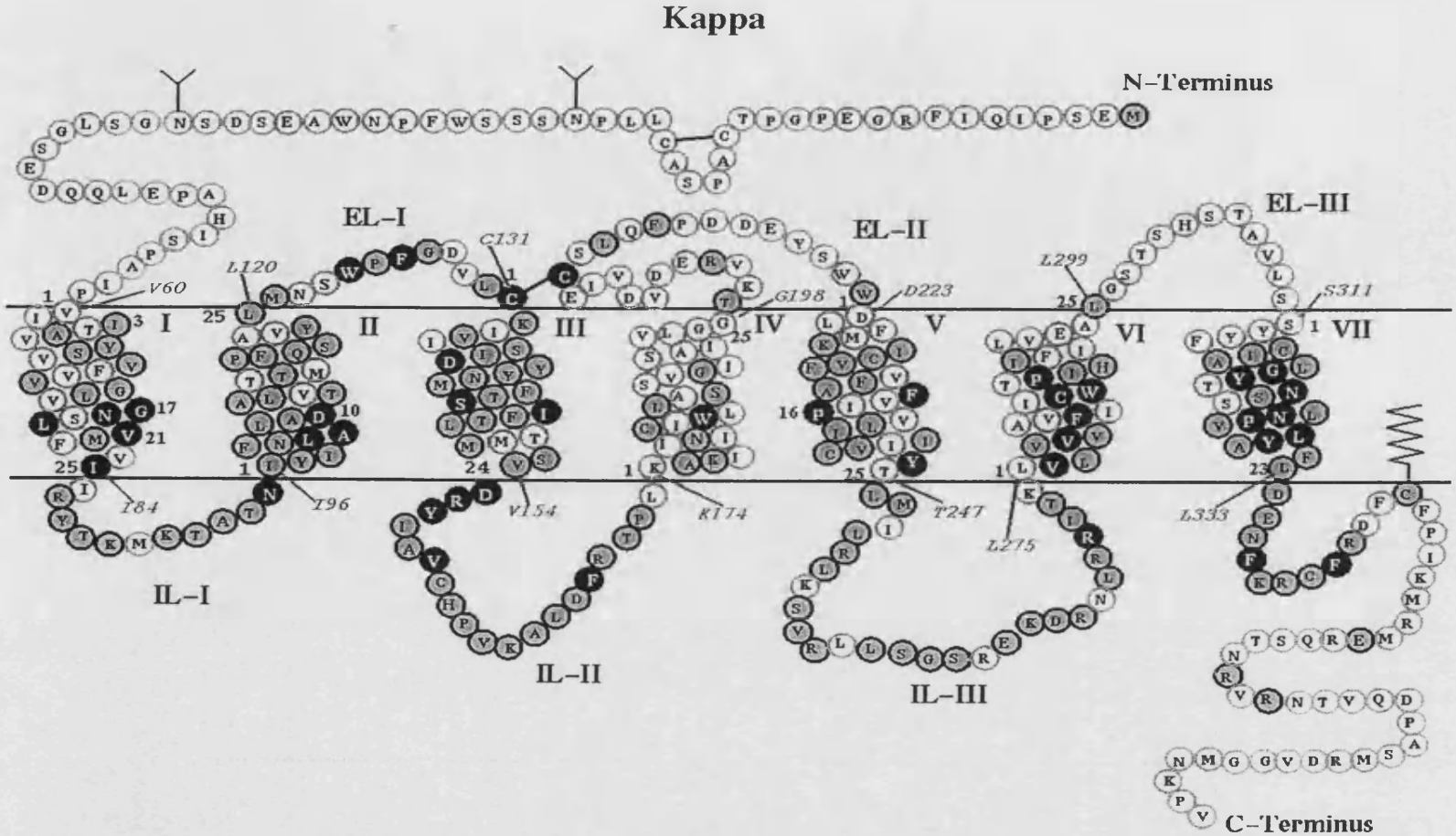
APPENDIX A

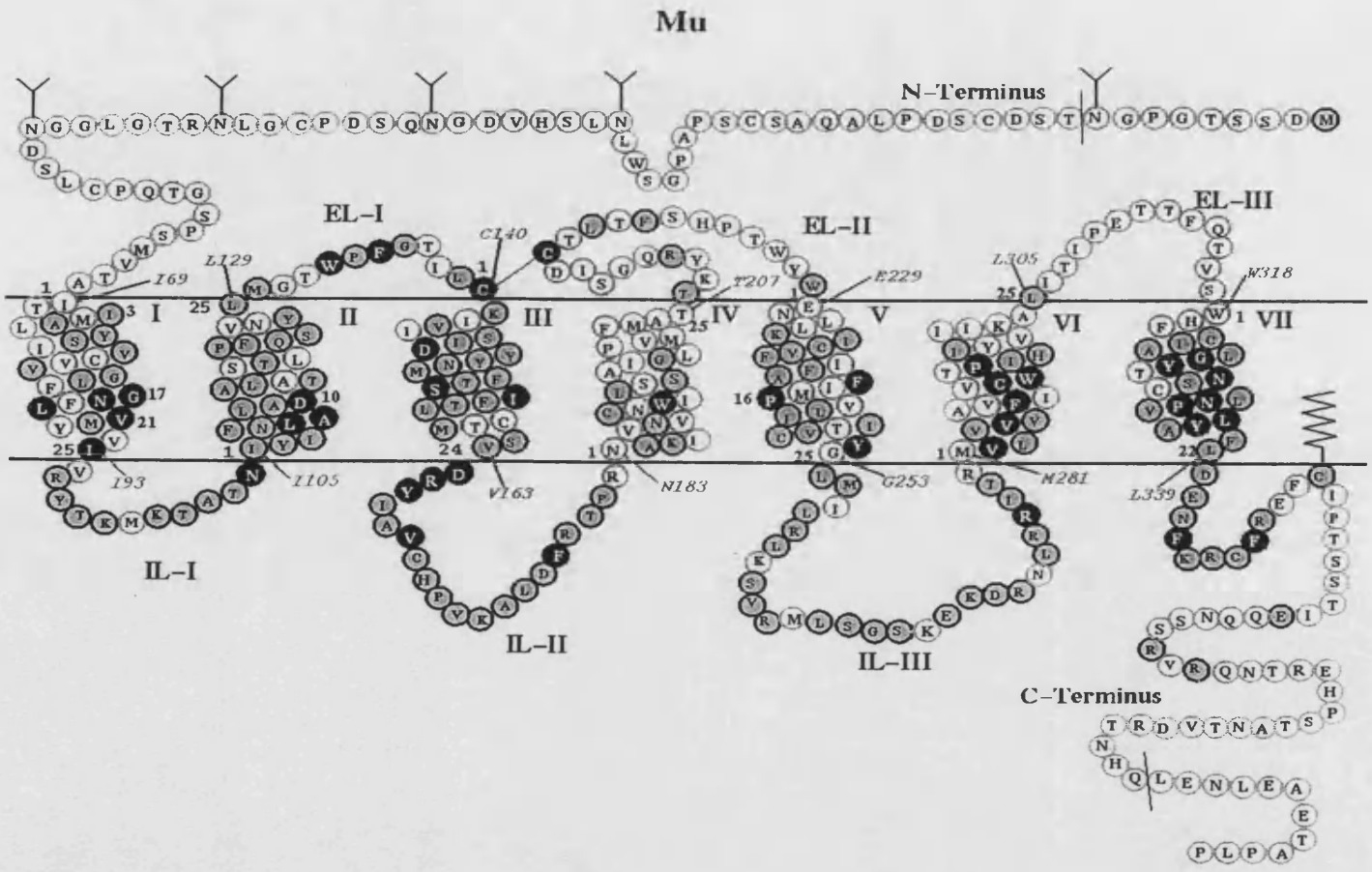
AMINO ACID NAMES AND STANDARD ABBREVIATIONS

Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic Acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic Acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

APPENDIX B

SCHEMATIC REPRESENTATION OF THE 3 OPIOID RECEPTOR TYPES[202]


Kappa
N-Terminus
EL-I
EL-II
EL-III
C131
L120
V60
G198
D223
L299
S311
I
II
III
IV
V
VI
VII
I84
T96
V154
K174
T247
L275
L333
IL-I
IL-II
IL-III
C-Terminus


Mu
N-Terminus
EL-I
EL-II
EL-III
I
II
III
IV
V
VI
VII
IL-I
IL-II
IL-III
C-Terminus
L129
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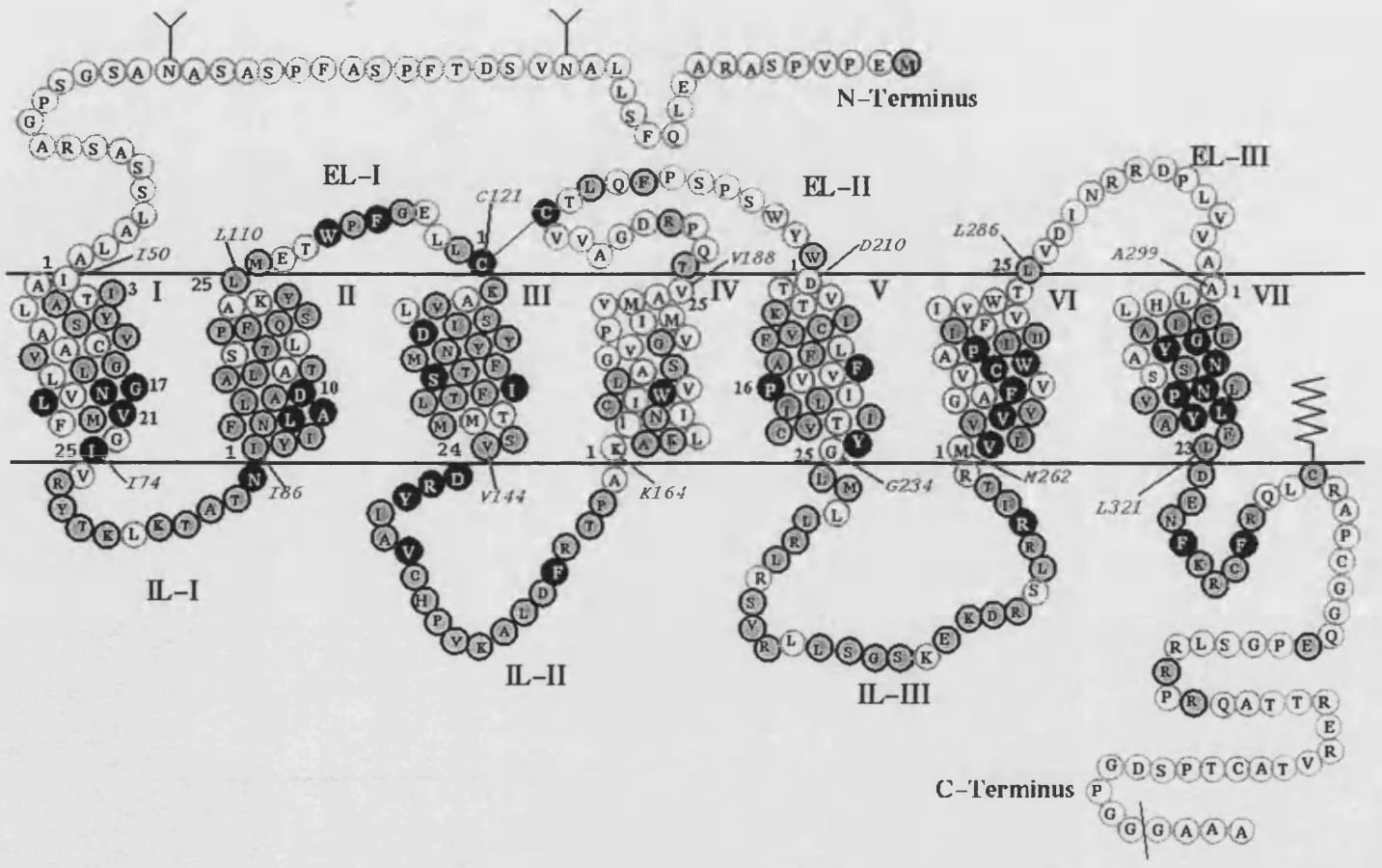
T207
E229
L305
W318
I69
I93
I105
V163
N183
G253
M281
L339

Delta


N-Terminus
EL-I
EL-II
EL-III
I
II
III
IV
V
VI
VII
L110
I150
C121
V188
D210
L286
A299
I74
I86
V144
K164
G234
M262
L321
IL-I
IL-II
IL-III
C-Terminus

APPENDIX C

PROCEDURE USED FOR MODELLING THE κ-OPIOID RECEPTOR

Using the known amino acid sequence of the kappa opioid receptor and the SYBYL package, COMPOSER,[184] the sequence of the kappa receptor was matched to homologous sequences in Bovine Rhodopsin (Brookhaven Protein Database, 1F88), as well as to a model of the δ-opioid receptor previously determined by Brandt.[189] Often this matching can be improved manually because of differing lengths of non-conserved regions in the proteins. COMPOSER then copies the structure of these conserved regions from the known receptor. The Brookhaven Protein Database is searched for sections which are similar in both sequence and length, to those which do not match the known receptor, and these are then used to propose the structure of the non-conserved portion of the protein. For the kappa receptor, the presence of the disulphide bond between extracellular loops 1 and 2 is well documented.[203] From the loop search, only those sequences in which the two cysteine molecules are in appropriate positions for the formation of a disulphide bridge can therefore be considered.

All minimisations were performed using the Tripos Force Field, Gasteiger-Marsili charges and a distance dependent dielectric function, with a dielectric constant of 4.0. The termination gradient was set at 0.05 kcal/mol with a minimum energy change of 0.05. The receptor structure was minimised and evaluated using the program, ProCheck.[185] This program determines whether the amino acid backbone and side chains are in feasible orientations (with respect to bond lengths, chirality, planarity, bond angles, torsion angles, etc).

The model was further validated by docking known κ-selective agonists and antagonists into the receptor, and comparing the interactions of the receptor/ligand complex to site directed mutagenesis studies already published.

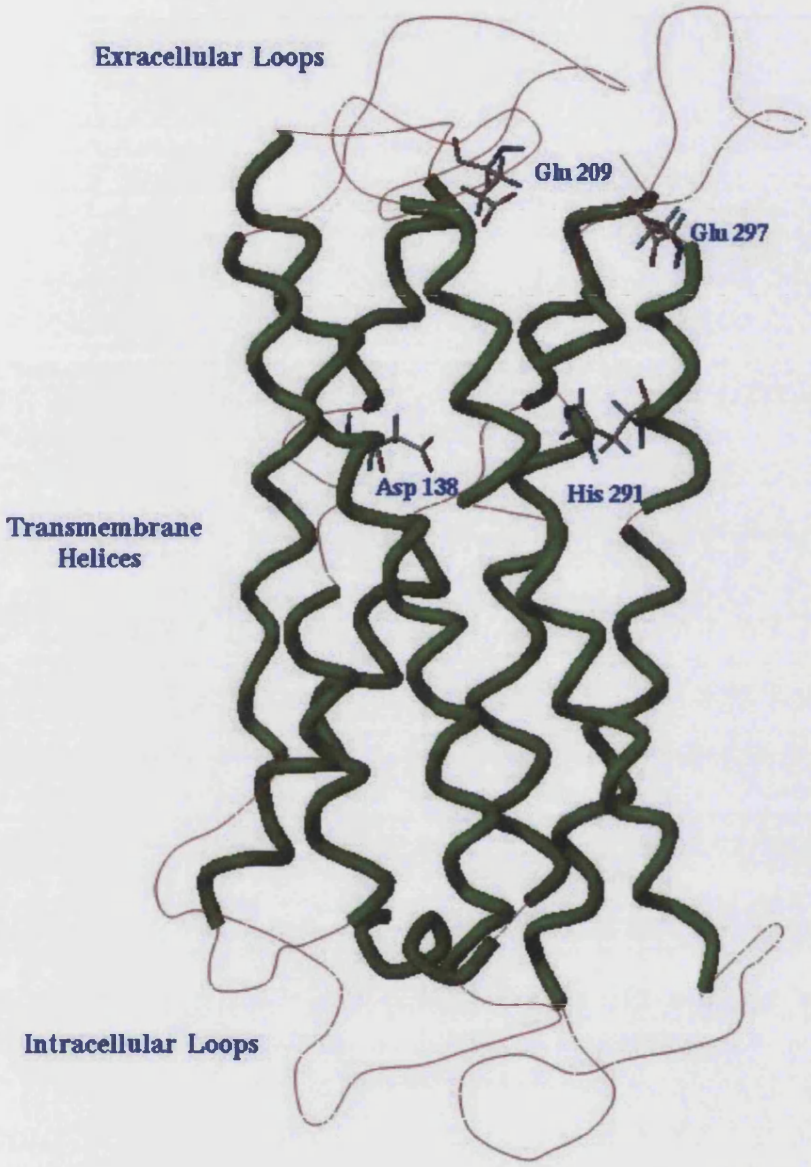
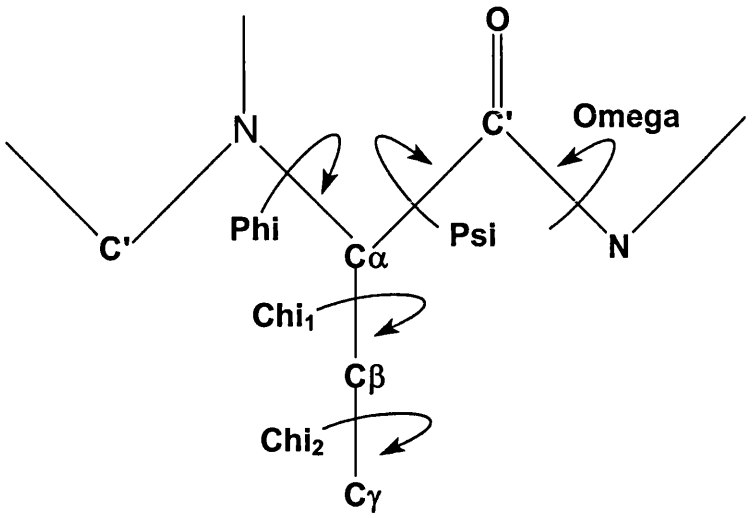


Fig. 27 Representation of the modelled κ-opioid receptor showing extracellular loops, transmembrane regions and intracellular loops.

APPENDIX D

PROCHECK ANALYSIS OF THE RECEPTOR

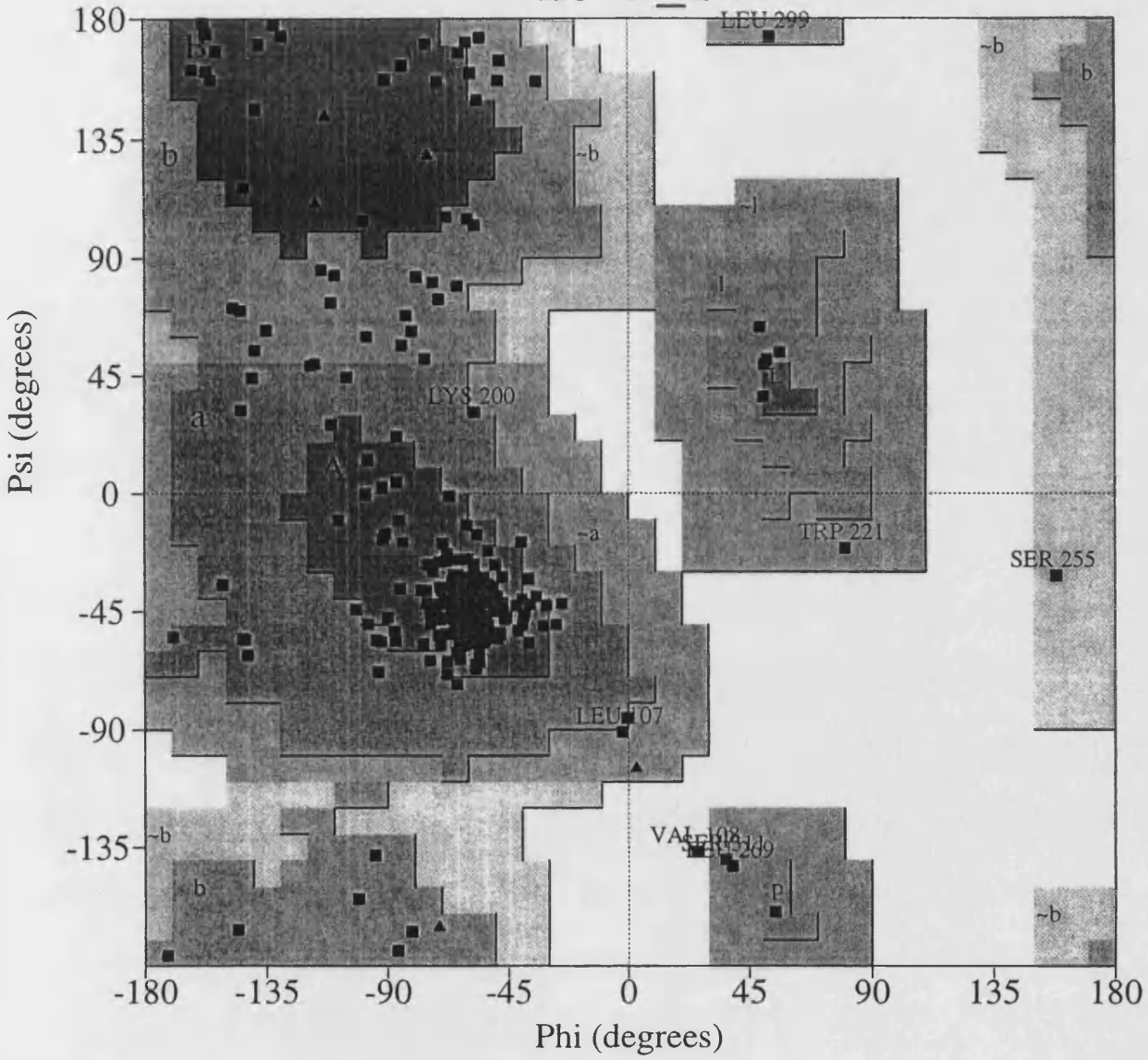


Phi	C' - N - C_A - C'
Psi	N - C_A - C' - N
Chi1	N - C_A - C_B - C_C
Chi2	C_A - C_B - C_C - C_D
Omega	C_A - C' - N - C_A

Fig. 1 Guide to Torsion Angles and Atom Numbering

Ramachandran Plot

nb42_1



Plot statistics

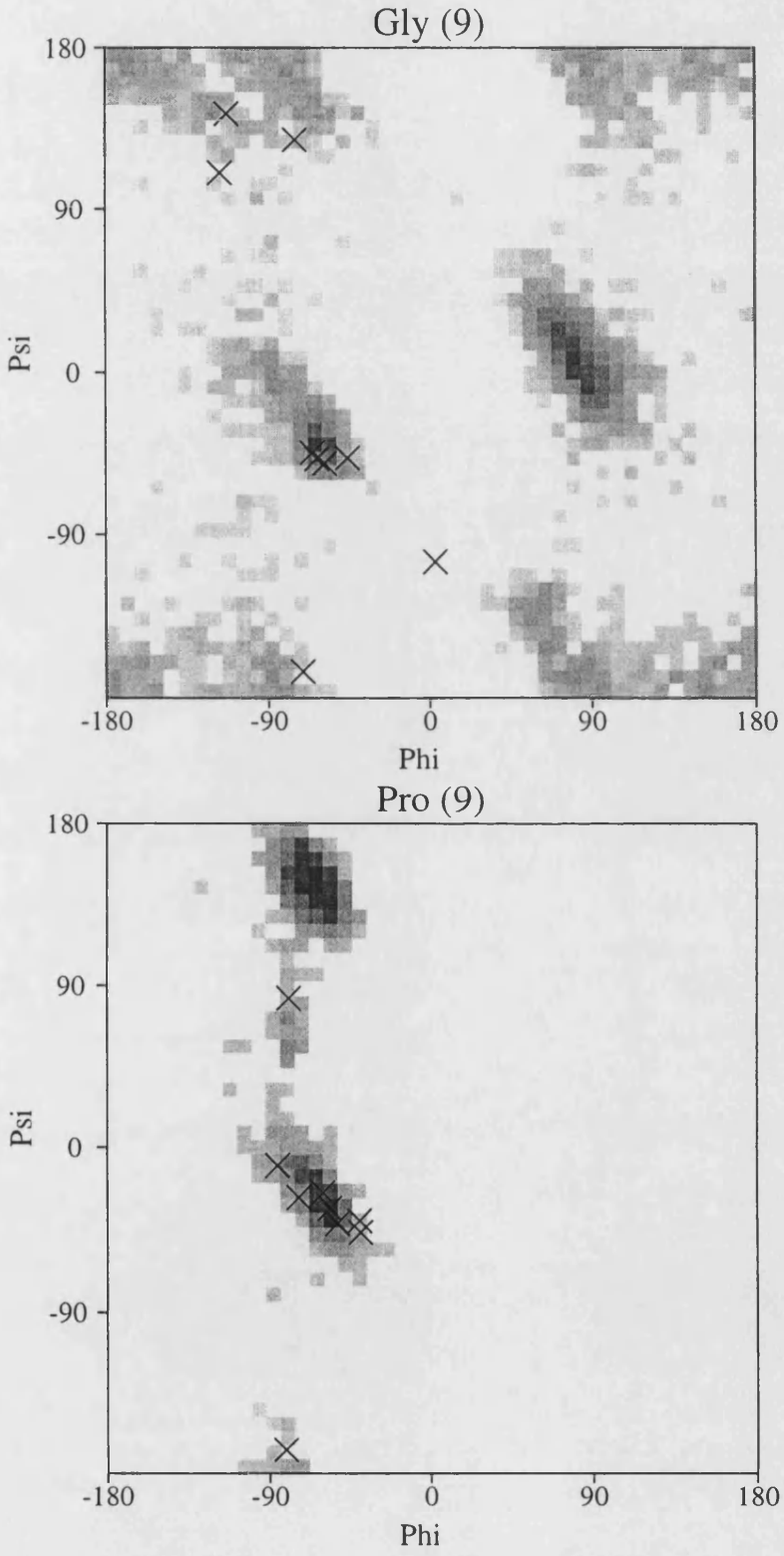
Residues in most favoured regions [A,B,L]	201	75.6%
Residues in additional allowed regions [a,b,l,p]	57	21.4%
Residues in generously allowed regions [~a,~b,~l,~p]	7	2.6%
Residues in disallowed regions	1	0.4%
	----	------
Number of non-glycine and non-proline residues	266	100.0%
Number of end-residues (excl. Gly and Pro)	2	
Number of glycine residues (shown as triangles)	9	
Number of proline residues	9	

Total number of residues	286	

Based on an analysis of 118 structures of resolution of at least 2.0 Angstroms and R-factor no greater than 20%, a good quality model would be expected to have over 90% in the most favoured regions.

Ramachandran plots for Gly & Pro

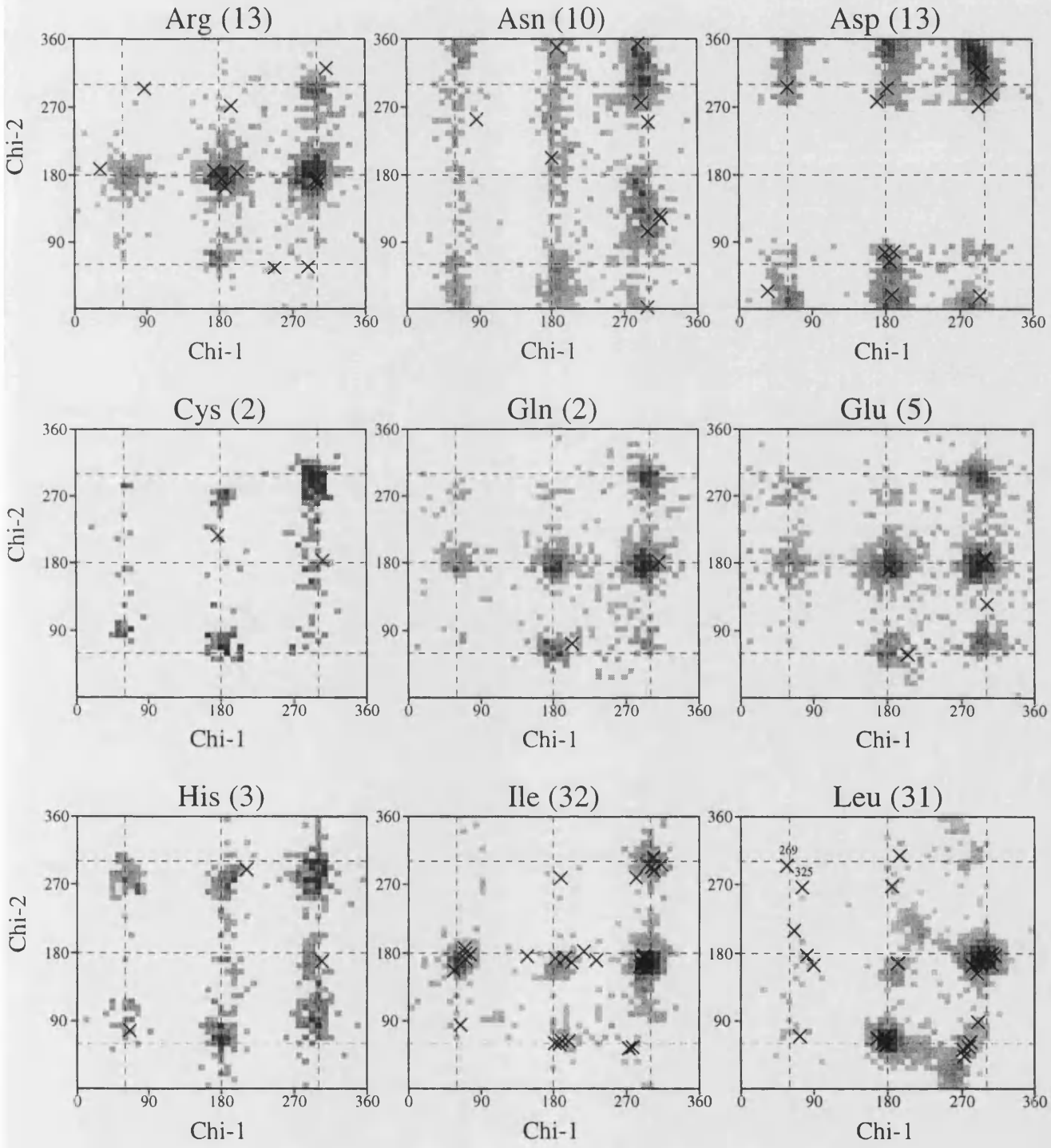
nb42_1



Numbers of residues are shown in brackets. Those in unfavourable conformations (score < -4.00) are labelled.
Shading shows favourable conformations as obtained from an analysis of 163 structures at resolution 2.0A or better.

Chi1-Chi2 plots

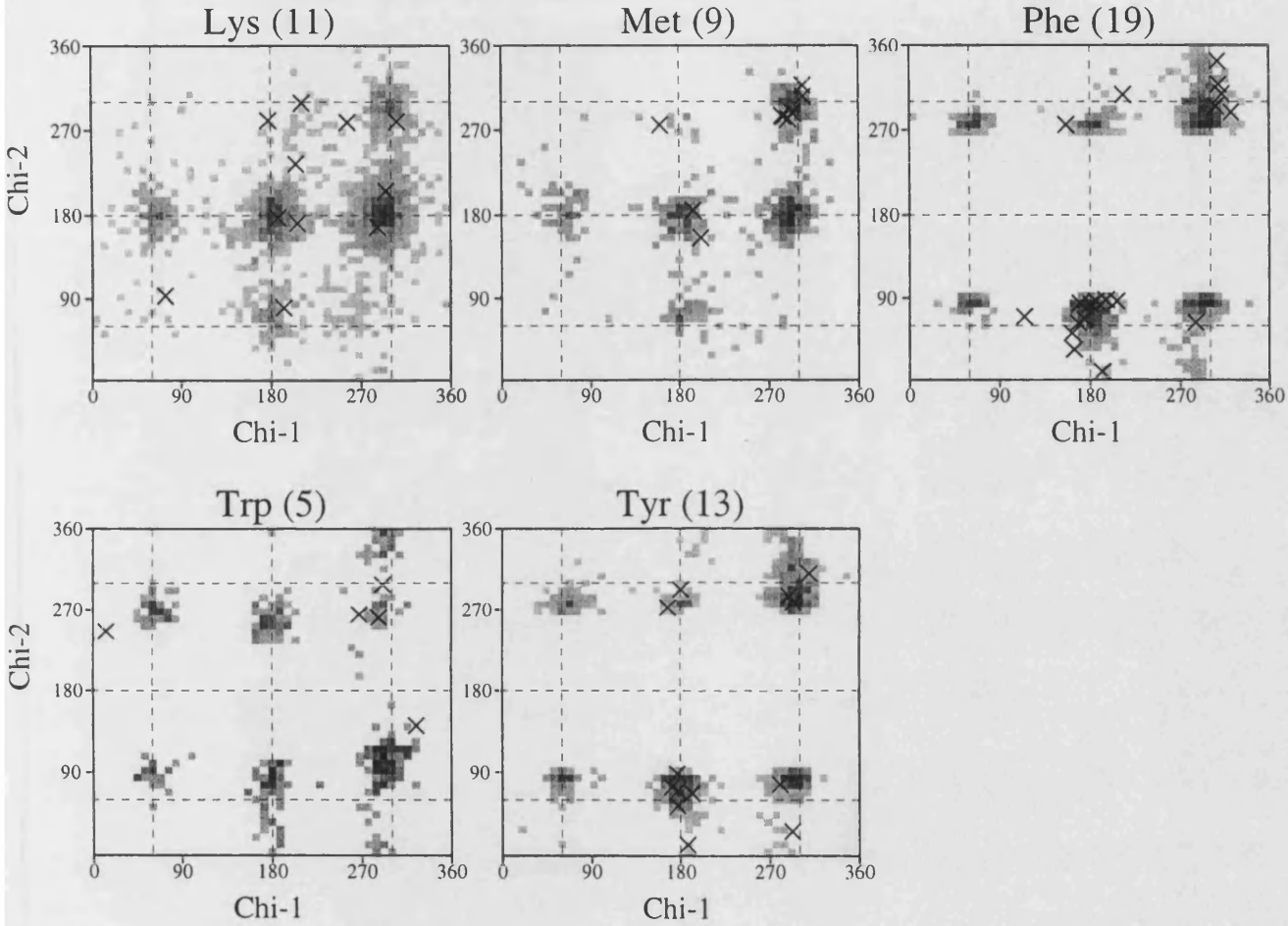
nb42_1



Numbers of residues are shown in brackets. Those in unfavourable conformations (score < -4.00) are labelled.
Shading shows favourable conformations as obtained from an analysis of 163 structures at resolution 2.0A or better.

Chi1-Chi2 plots

nb42_1

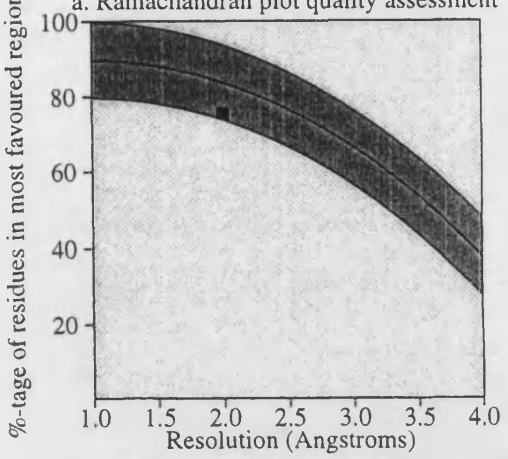


Numbers of residues are shown in brackets. Those in unfavourable conformations (score < -4.00) are labelled.
Shading shows favourable conformations as obtained from an analysis of 163 structures at resolution 2.0A or better.

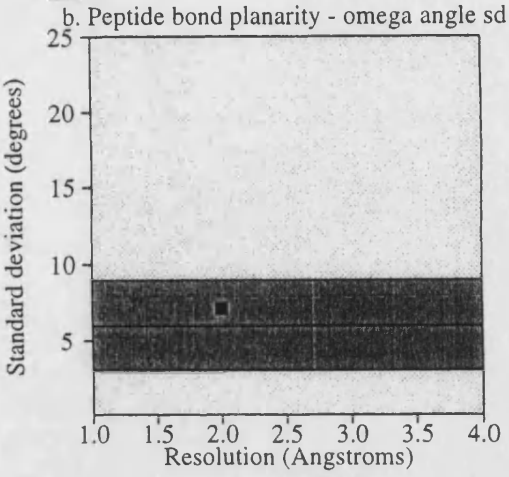
Main-chain parameters

nb42_1

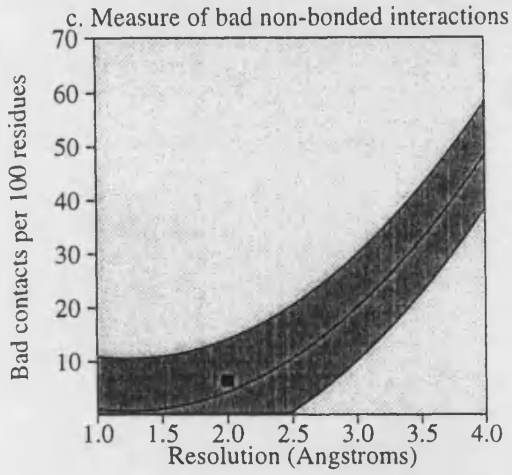
a. Ramachandran plot quality assessment



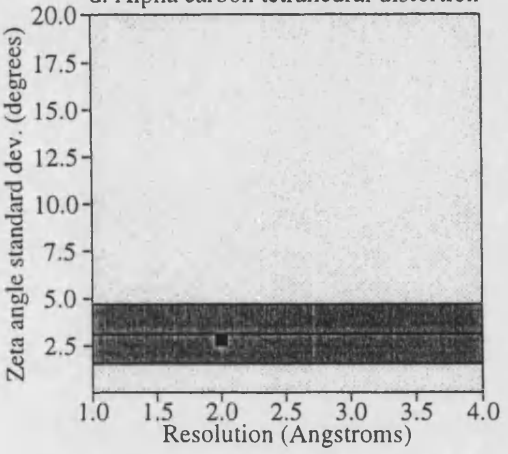
b. Peptide bond planarity - omega angle sd



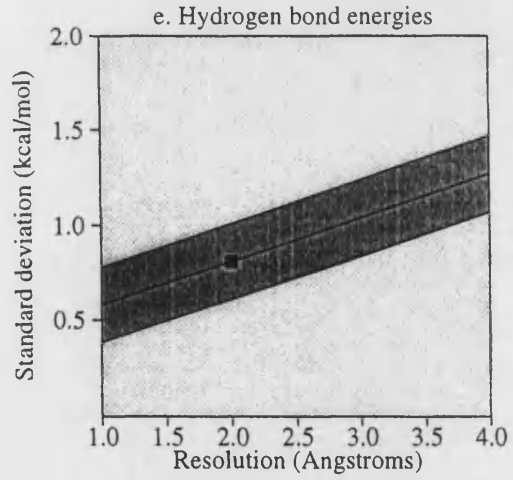
c. Measure of bad non-bonded interactions



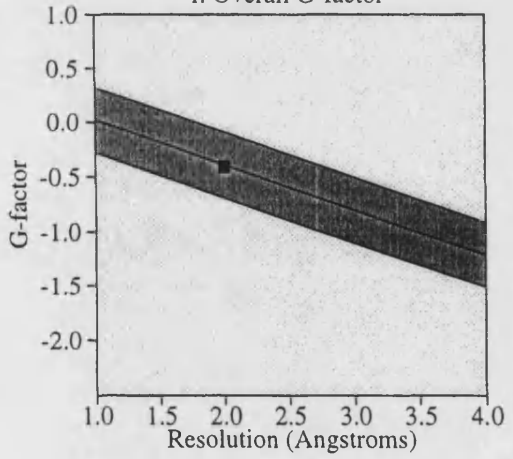
d. Alpha carbon tetrahedral distortion



e. Hydrogen bond energies



f. Overall G-factor

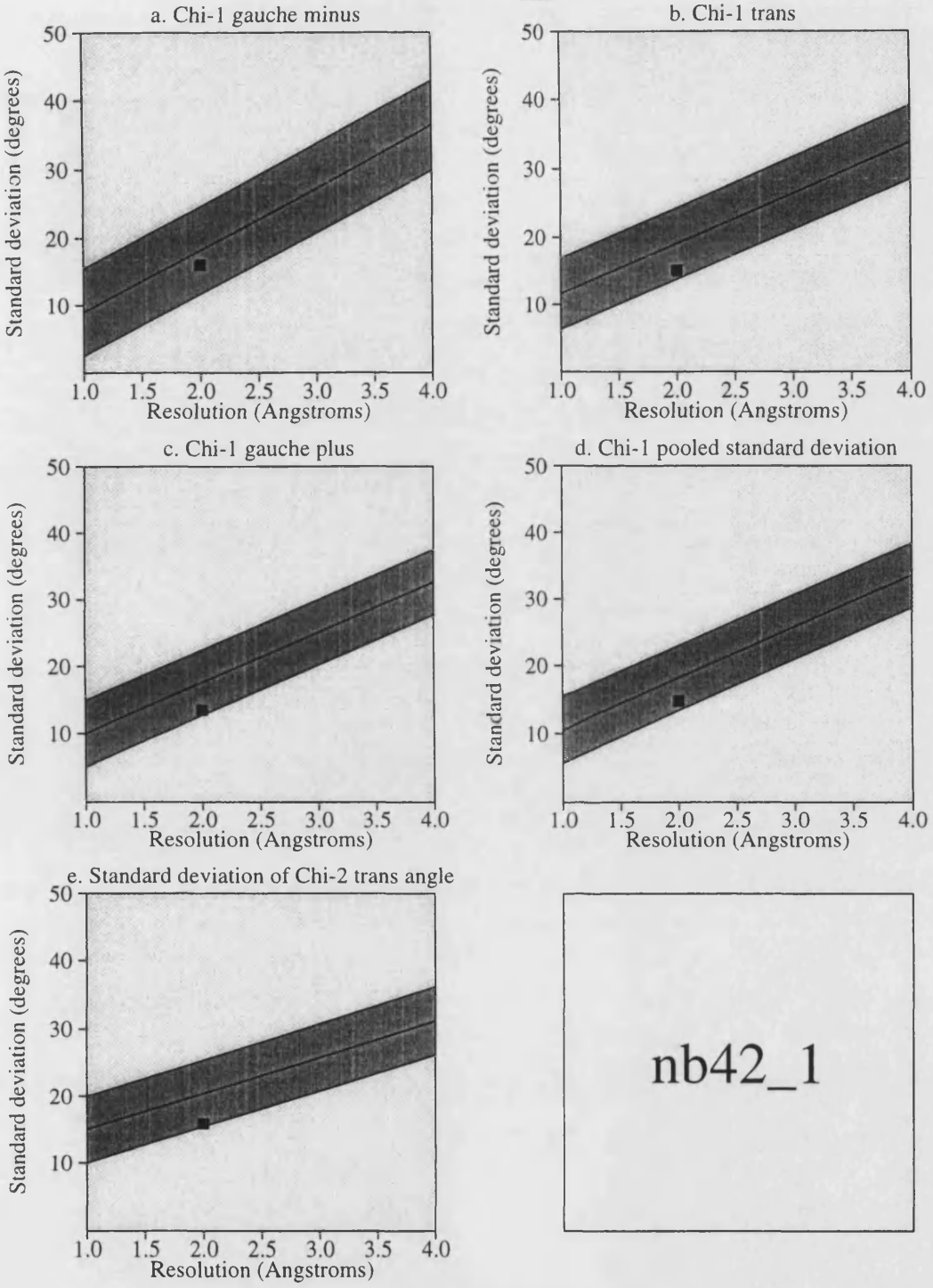


Plot statistics

Stereochemical parameter	No. of data pts	Parameter value	Comparison values Typical value	Band width	No. of band widths from mean	
a. %-tage residues in A, B, L	266	75.6	83.8	10.0	-0.8	Inside
b. Omega angle st dev	285	7.1	6.0	3.0	0.4	Inside
c. Bad contacts / 100 residues	18	6.3	4.2	10.0	0.2	Inside
d. Zeta angle st dev	277	2.8	3.1	1.6	-0.2	Inside
e. H-bond energy st dev	203	0.8	0.8	0.2	0.0	Inside
f. Overall G-factor	286	-0.4	-0.4	0.3	-0.1	Inside

Side-chain parameters

nb42_1

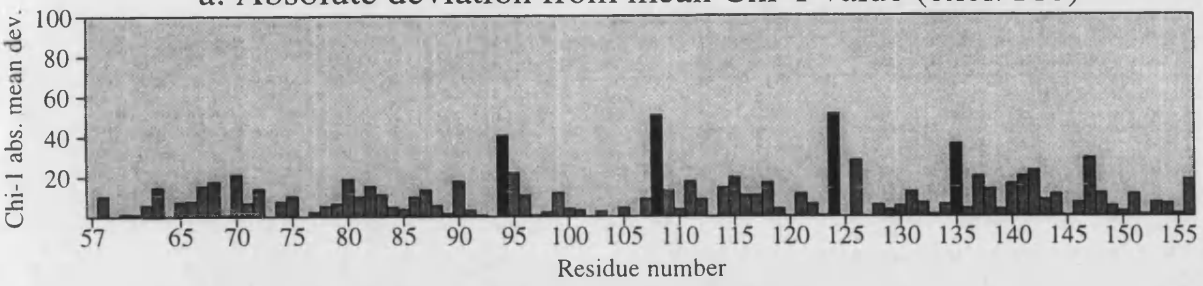


Plot statistics

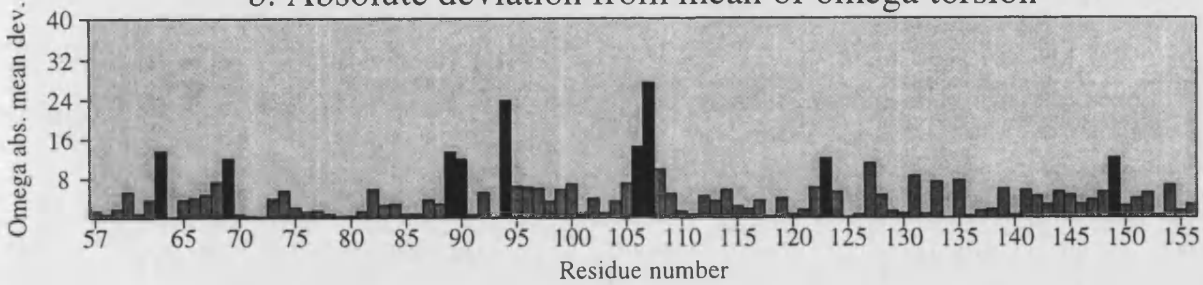
Stereochemical parameter	No. of data pts	Parameter value	Comparison values Typical value	Band width	No. of band widths from mean	
a. Chi-1 gauche minus st dev	45	16.0	18.1	6.5	-0.3	Inside
b. Chi-1 trans st dev	100	15.0	19.0	5.3	-0.8	Inside
c. Chi-1 gauche plus st dev	106	13.4	17.5	4.9	-0.8	Inside
d. Chi-1 pooled st dev	251	14.7	18.2	4.8	-0.7	Inside
e. Chi-2 trans st dev	55	15.8	20.4	5.0	-0.9	Inside

Residue properties
nb42_1

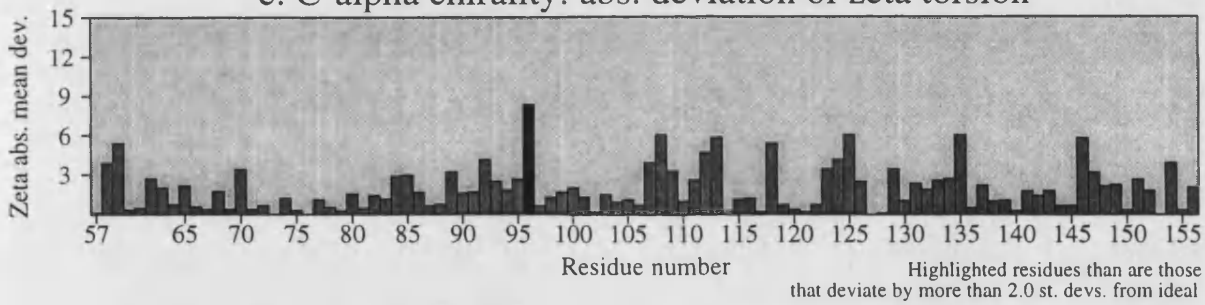
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion



c. C-alpha chirality: abs. deviation of zeta torsion

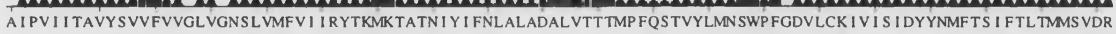


Highlighted residues than are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility

Key:- Helix Beta strand Random coil Accessibility shading: Black=buried, White=accessible

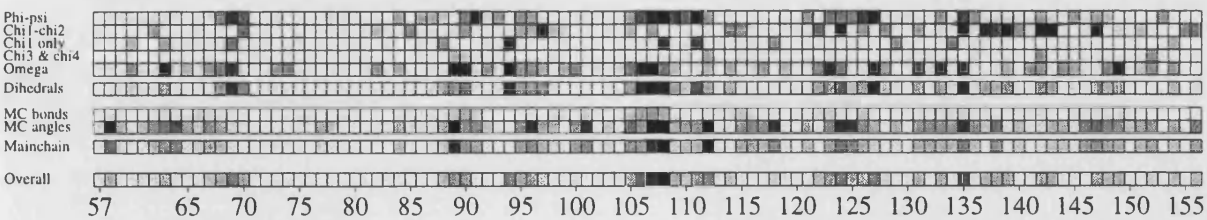
e. Sequence & Ramachandran regions ▲ Most favoured ■ Allowed ▮ Generous ▮ Disallowed



AIPVIITAVYSVVFVVGLVGNSLVMFVIIRYTKMKTATNIYIFNLALADALVTTTMPFQSTVYLMNSWPFGDVLCKIVISIDYYNMFTSIFTLTMMSVDR

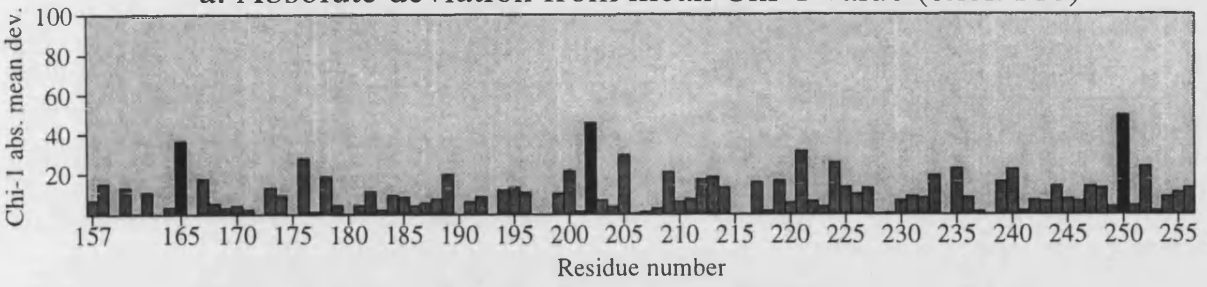
f. Max. deviation (see listing)

g. G-factors

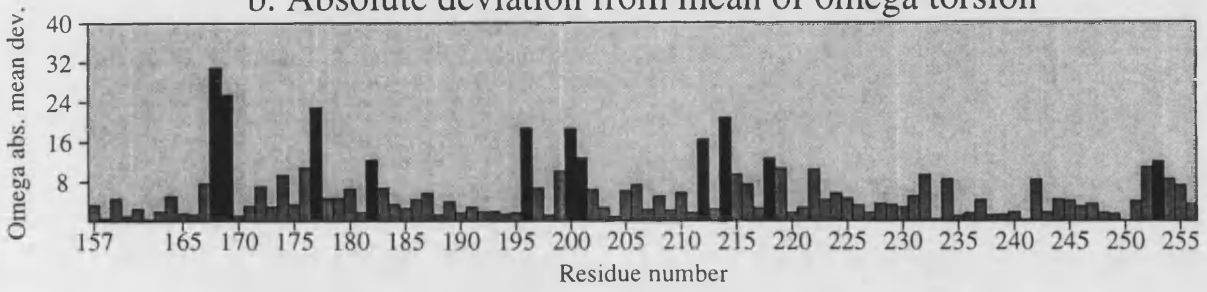


Residue properties
nb42_1

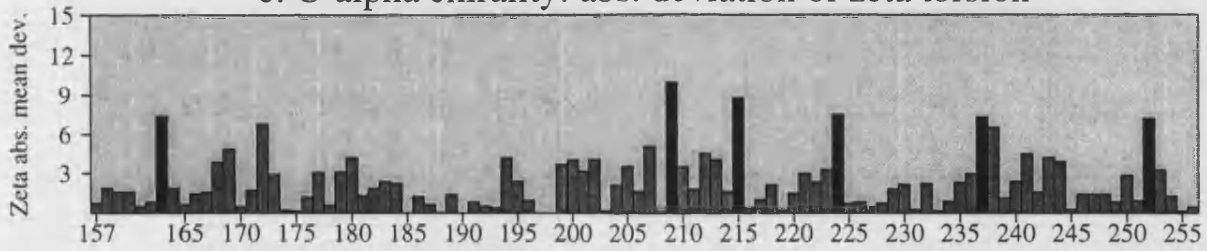
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion



c. C-alpha chirality: abs. deviation of zeta torsion

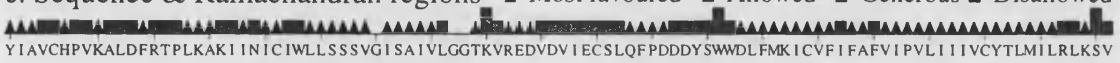


Highlighted residues than are those that deviate by more than 2.0 st. devs. from ideal

d. Secondary structure & estimated accessibility

Key:- Helix Beta strand Random coil Accessibility shading: Black=buried, White=accessible

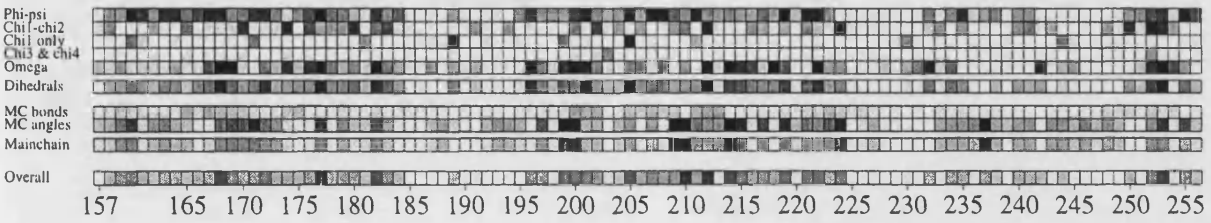
e. Sequence & Ramachandran regions ▲ Most favoured ■ Allowed ▮ Generous ▮ Disallowed



YIAVCHPVKALDFRTPLKAKIINICIWLLSSSVGISAIVLGGTKVREDVDVIECSLQFPDDDYSWWDLFMKICVFIFAFVIPVLIIIVCYTLMILRLKSV

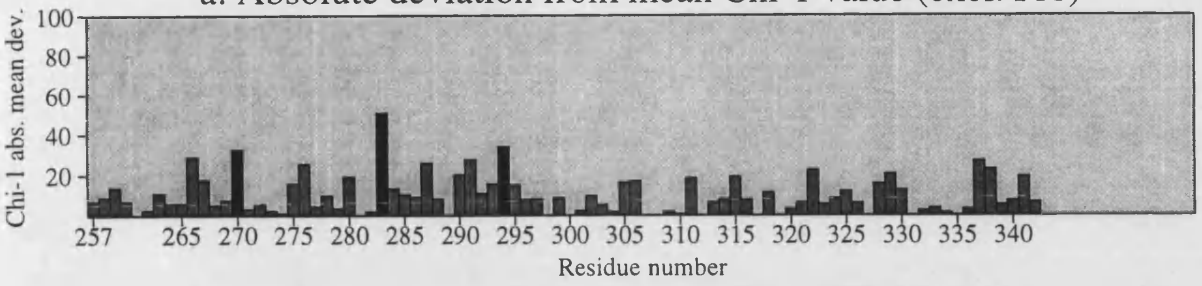
f. Max. deviation (see listing)

g. G-factors

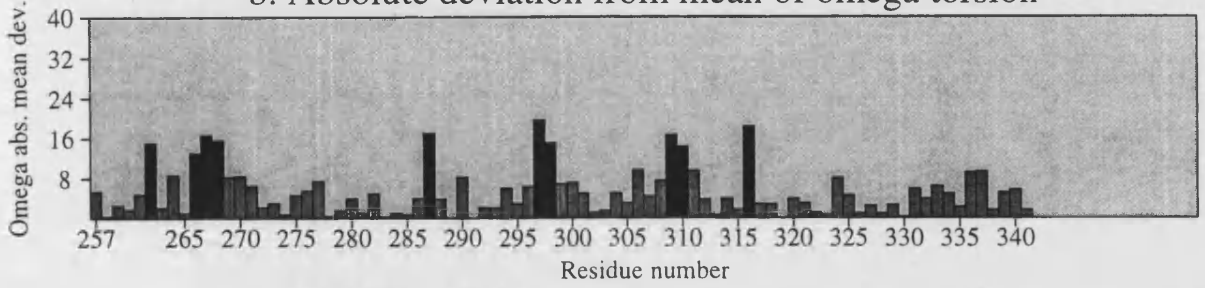


Residue properties
nb42_1

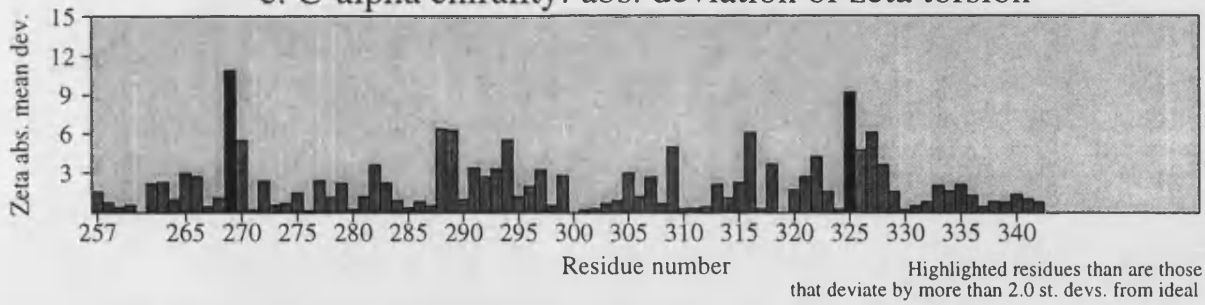
a. Absolute deviation from mean Chi-1 value (excl. Pro)



b. Absolute deviation from mean of omega torsion



c. C-alpha chirality: abs. deviation of zeta torsion



Highlighted residues than are those that deviate by more than 2.0 st. devs. from ideal

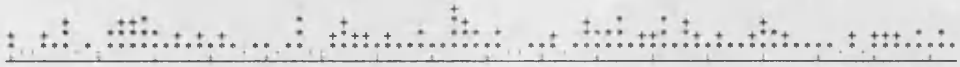
d. Secondary structure & estimated accessibility

Key:- Helix Beta strand Random coil Accessibility shading: Black=buried, White=accessible

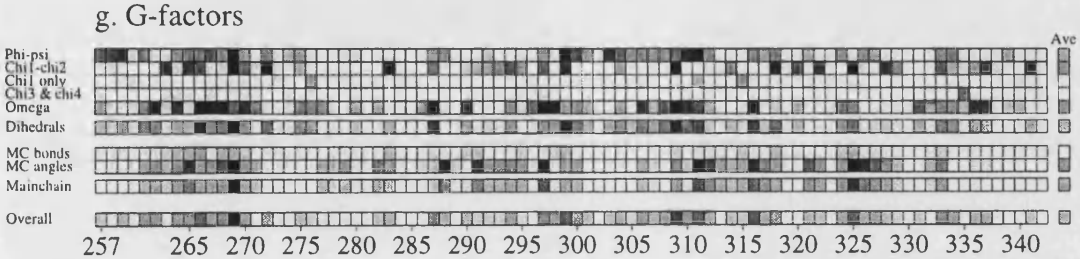
e. Sequence & Ramachandran regions ▲ Most favoured ■ Allowed ▮ Generous ▮ Disallowed

RLLSGSREKDRNLRRITRLVLVVVAVFVVCWTPIHIFILVEALGSTSHSTAALSSYYFCIALGYTNSSLNPILYAFLDENFKRCFR

f. Max. deviation (see listing)

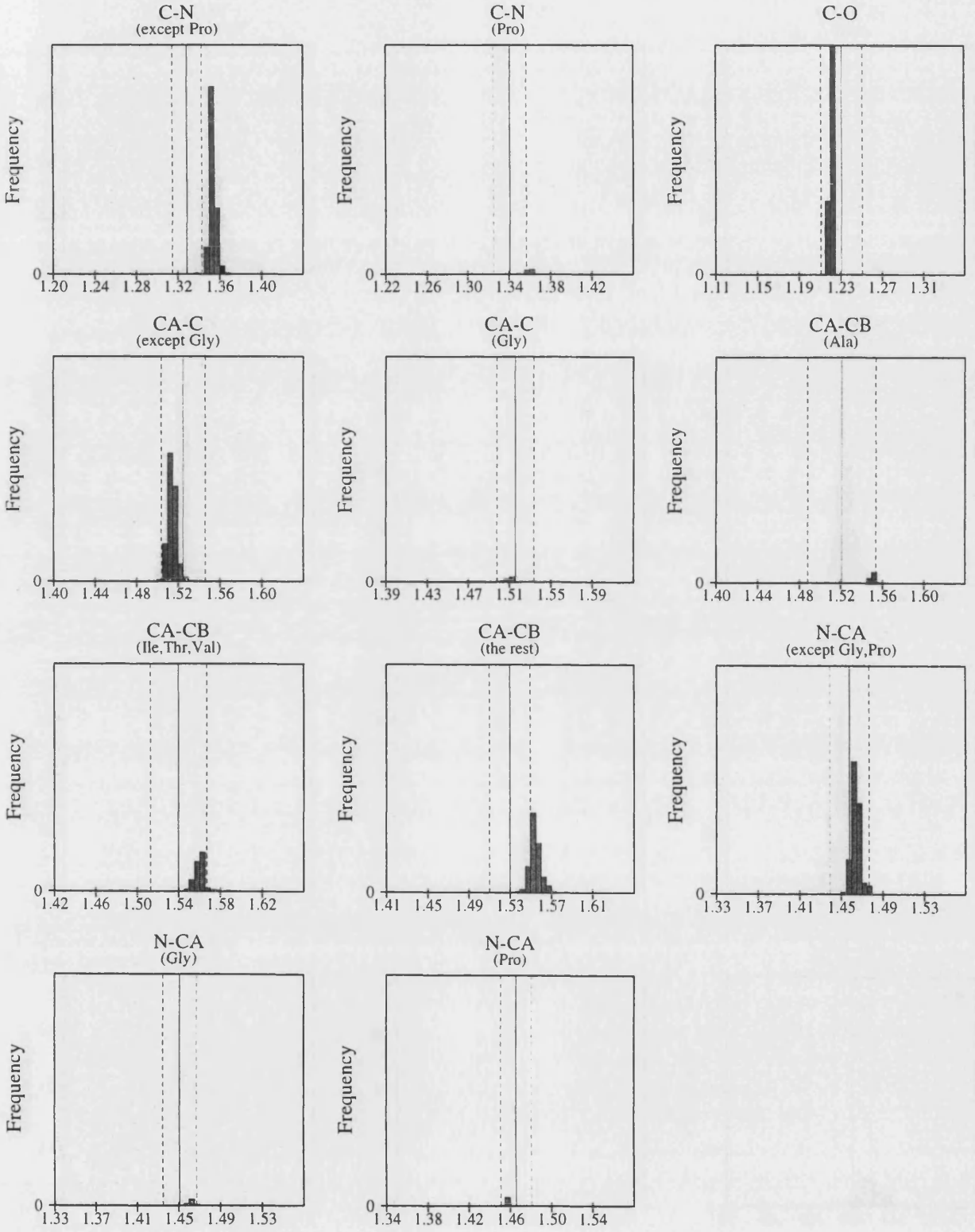


g. G-factors



Main-chain bond lengths

nb42_1

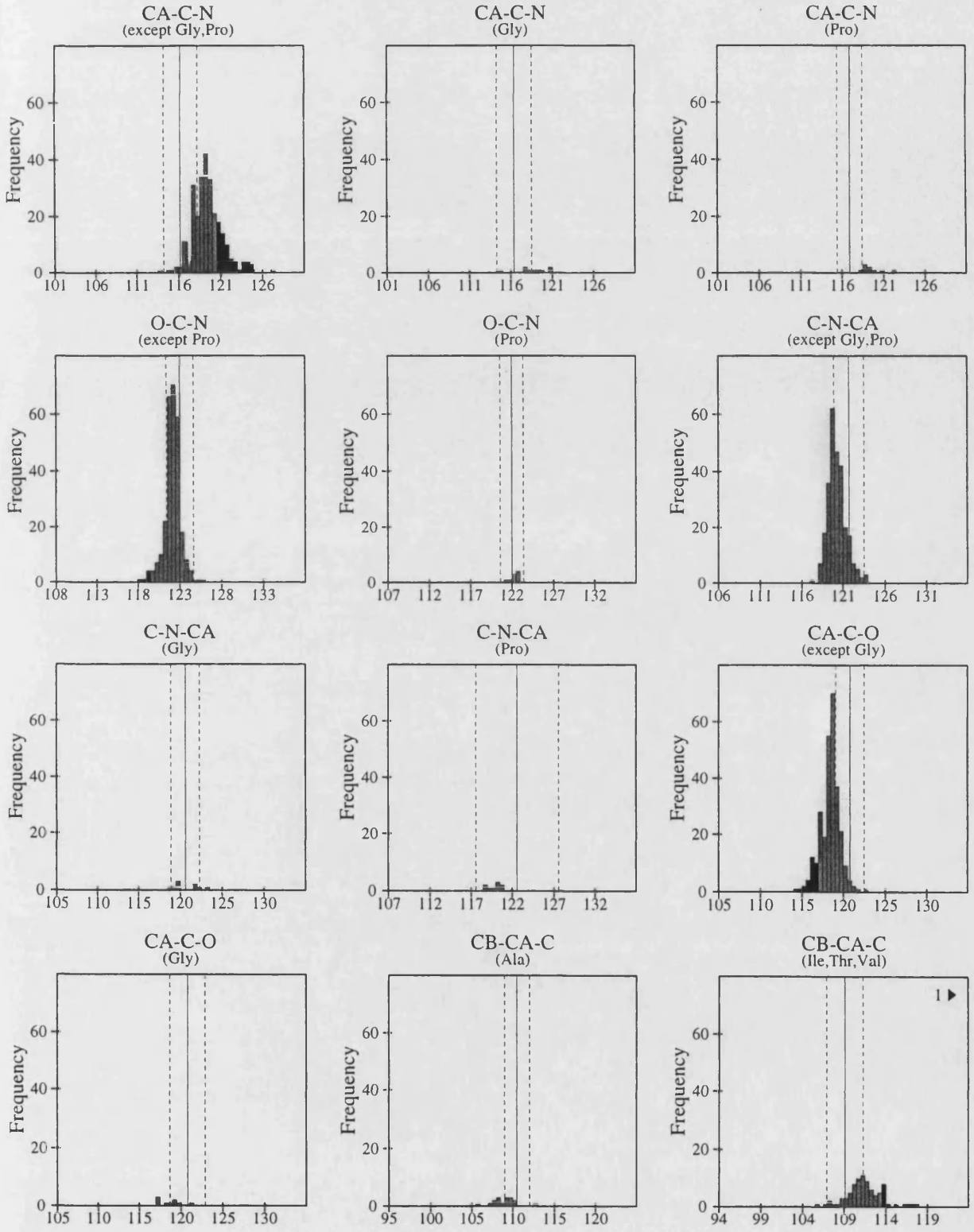


Black bars > 2.0 st. devs. from mean.

Solid and dashed lines represent the mean and standard deviation values as per Engh & Huber small-molecule data.

Main-chain bond angles

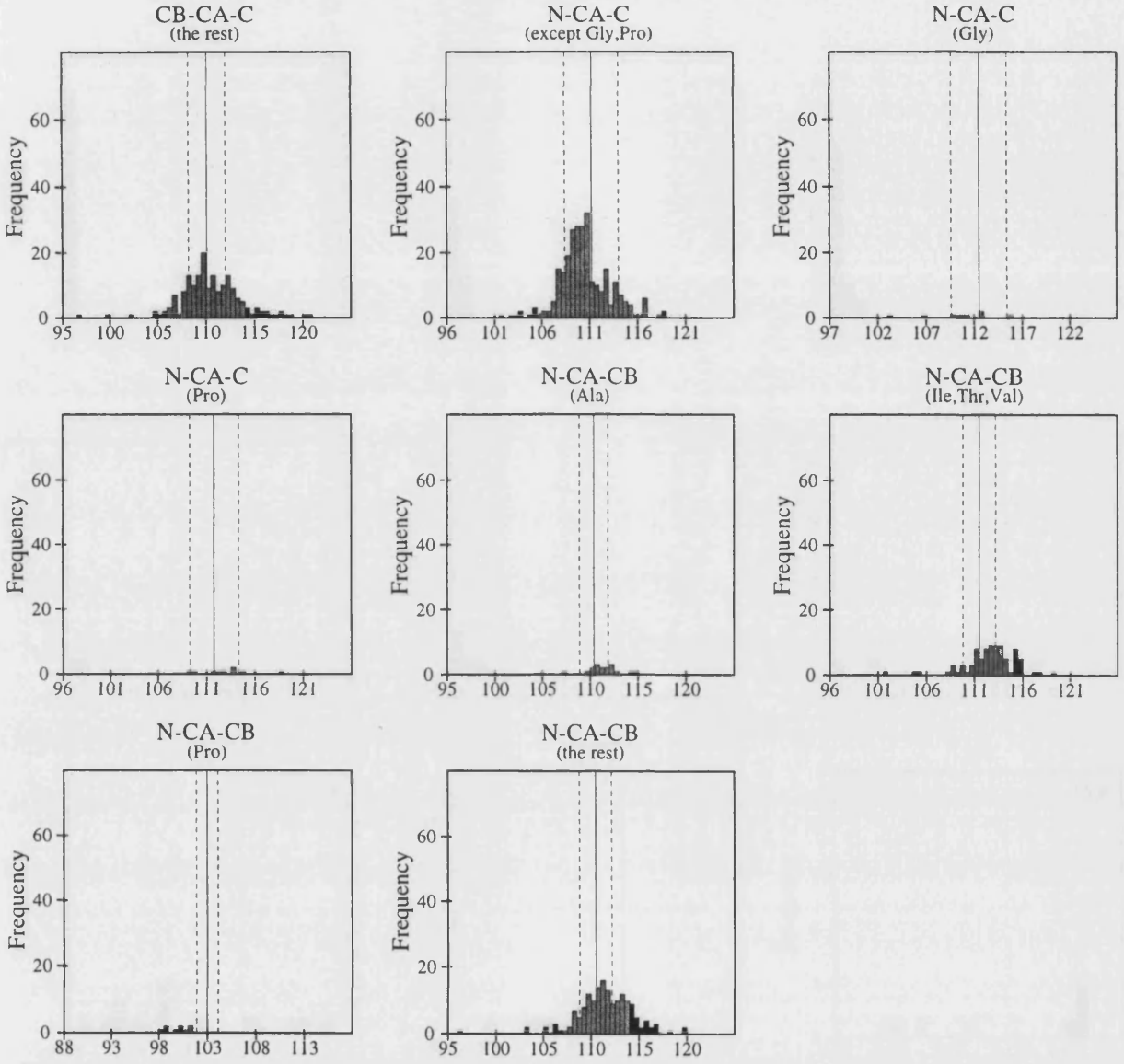
nb42_1



Black bars > 2.0 st. devs. from mean. ◀ or ▶ signifies data points off the graph in the direction shown.
Solid and dashed lines represent the mean and standard deviation values as per Engh & Huber small-molecule data.

Main-chain bond angles

nb42_1

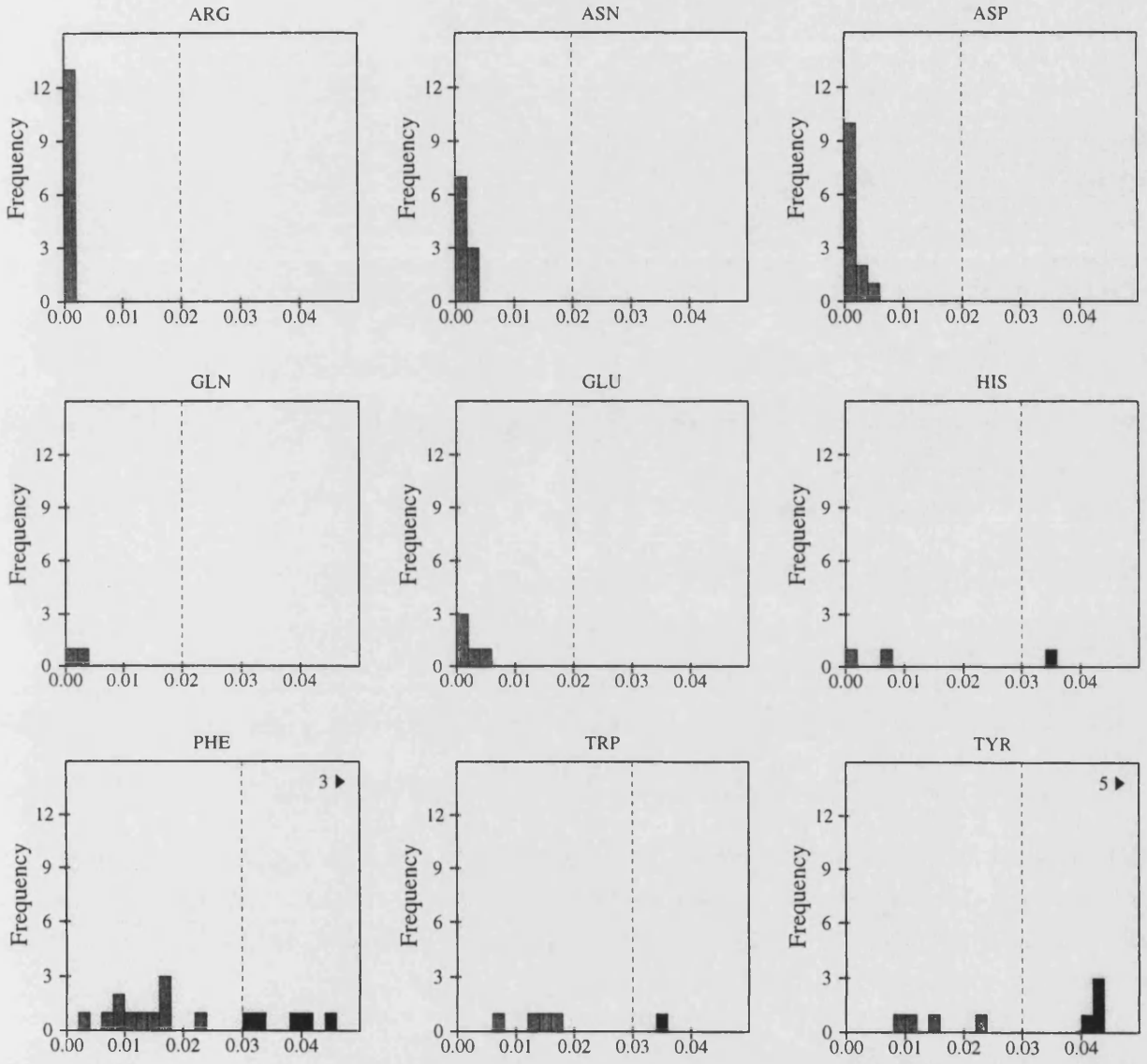


Black bars > 2.0 st. devs. from mean.

Solid and dashed lines represent the mean and standard deviation values as per Engh & Huber small-molecule data.

RMS distances from planarity

nb42_1

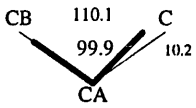


Histograms showing RMS distances of planar atoms from best-fit plane.
Black bars indicate large deviations from planarity: RMS dist > 0.03 for rings, and > 0.02 otherwise.

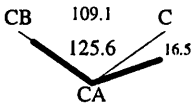
▶ signifies data points off the graph in the direction shown.

Distorted geometry
nb42_1

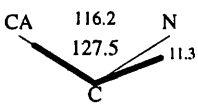
Main-chain bond angles



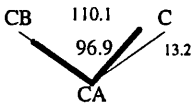
Lys 89



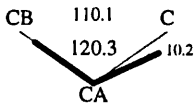
Val 108



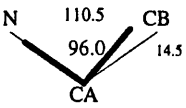
Met 112



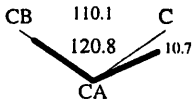
Glu 209



Cys 210



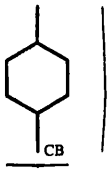
Cys 210



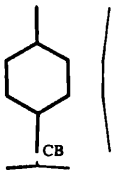
Leu 269

Bond angles differing by > 10.0 degrees from small-molec values. Values shown: "ideal", actual, diff.

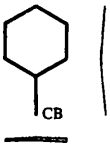
Planar groups



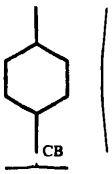
0.043
Tyr 66



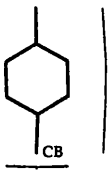
0.111
Tyr 97



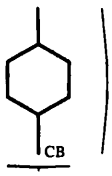
0.069
Phe 99



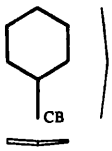
0.082
Tyr 119



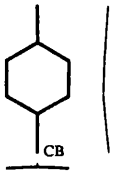
0.044
Tyr 139



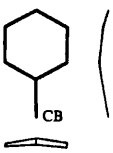
0.078
Tyr 140



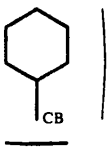
0.096
Phe 147



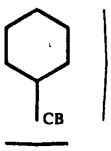
0.073
Tyr 157



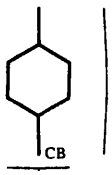
0.139
Phe 214



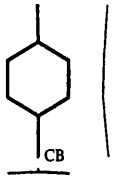
0.044
Phe 225



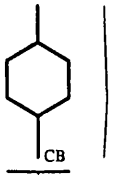
0.041
Phe 233



0.044
Tyr 312



0.073
Tyr 313



0.042
Tyr 330

Sidechains with RMS dist. from planarity > 0.04A for rings, or > 0.03A otherwise. Value shown is RMS dist.

APPENDIX E

FULL ASSIGNMENT OF A TYPICAL NMR

5'-Cyanomethyl-17-cyclopropylmethyl-6,7-didehydro-4,5α-epoxy-3,14-dihydroxyindolo-[2',3':6,7]morphinan (75)

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.18-0.23 [m, 2H, $NCH_2CH(CHHCHH)$], 0.57-0.65 [m, 2H, $NCH_2CH(CHHCHH)$], 0.89-0.97 [m, 1H, $NCH_2CH(CH_2CH_2)$], 1.16-1.19 [m, 2H, CH_2CN], 1.73-1.80 [m, 1H, C(8)H_aH_b], 2.28-2.38 [m, 1H, C(16)H_aH_b], 2.45-2.52 [m, 1H, C(8)H_aH_b], 2.61-2.66 [m, 2H, C(18)H_2], 2.78-2.84 [m, 1H, C(16)H_aH_b], 2.88-2.90 [m, 2H, C(15)H_2], 3.11-3.17 [m, 1H, C(10)H_aH_b], 3.44-3.46 [m, 1H, C(9)*H*], 3.76-3.78 [m, 1H, C(10)H_aH_b], 5.62 [s, 1H, C(5)*H*], 6.54 [d, J=8.2 Hz, 1H, C(1)*H*], 6.62 [d, J=8.2 Hz, 1H, C(2)*H*], 6.97 [d, J=8.6 Hz, 1H, C(6')*H*], 7.23 [d, J=8.6 Hz, 1H, C(7')*H*] and 7.28-7.32 [m, 1H, *C*(4')*H*]; ^{13}C NMR ($CDCl_3$) δ 3.8 [$NCH_2CH(CH_2CH_2)$], 4.3 [$NCH_2CH(CH_2CH_2)$], 9.2 [$NCH_2CH(CH_2CH_2)$], 23.5 [*C*(10)], 24.9 (CH_2CN), 28.9 [*C*(15)], 31.4 [*C*(8)], 44.1 [*C*(16)], 49.9 [quaternary *C*(13)], 59.5 [*C*(18)], 62.5 [*C*(9)], 73.1 [quaternary *C*(14)], 84.9 [*C*(5)], 112.2 [*C*(7')], 117.4 (*C*N), 118.3 [*C*(2)], 119.1 [*C*(4')], 119.3 [*C*(1)], 120.2 [*C*(5')], 122.3 [*C*(6')], 124.5 [quaternary *C*(11)], 128.1 [*C*(9')], 128.2 [*C*(7)], 130.7 [quaternary *C*(12)], 130.9 [*C*(8')], 137.0 [quaternary *C*(3)], 139.9 [quaternary *C*(6)], and 143.5 [quaternary *C*(4)].

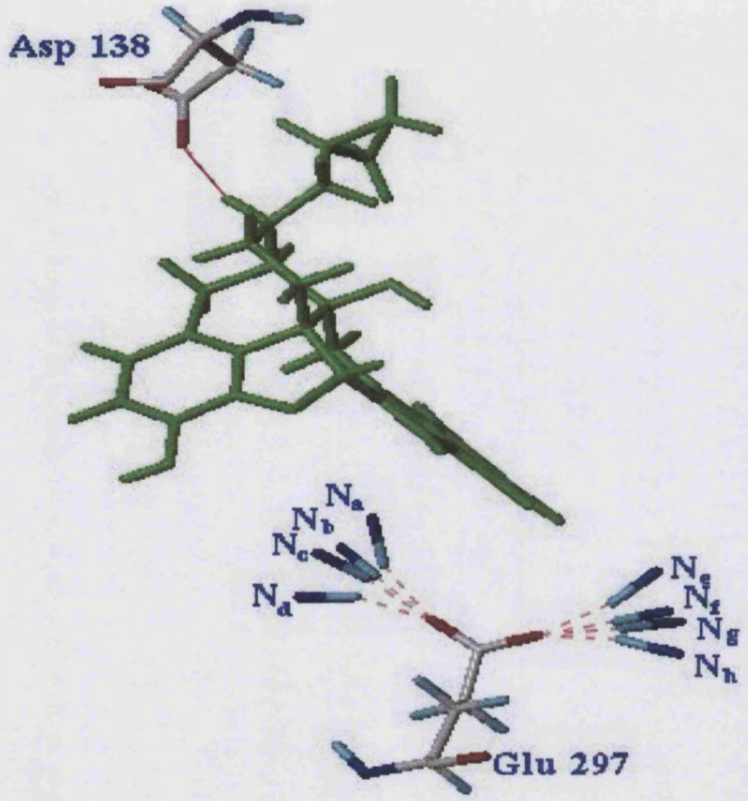


Fig. 4 Possible positions of NH groups able to form hydrogen bonds with Glu 297

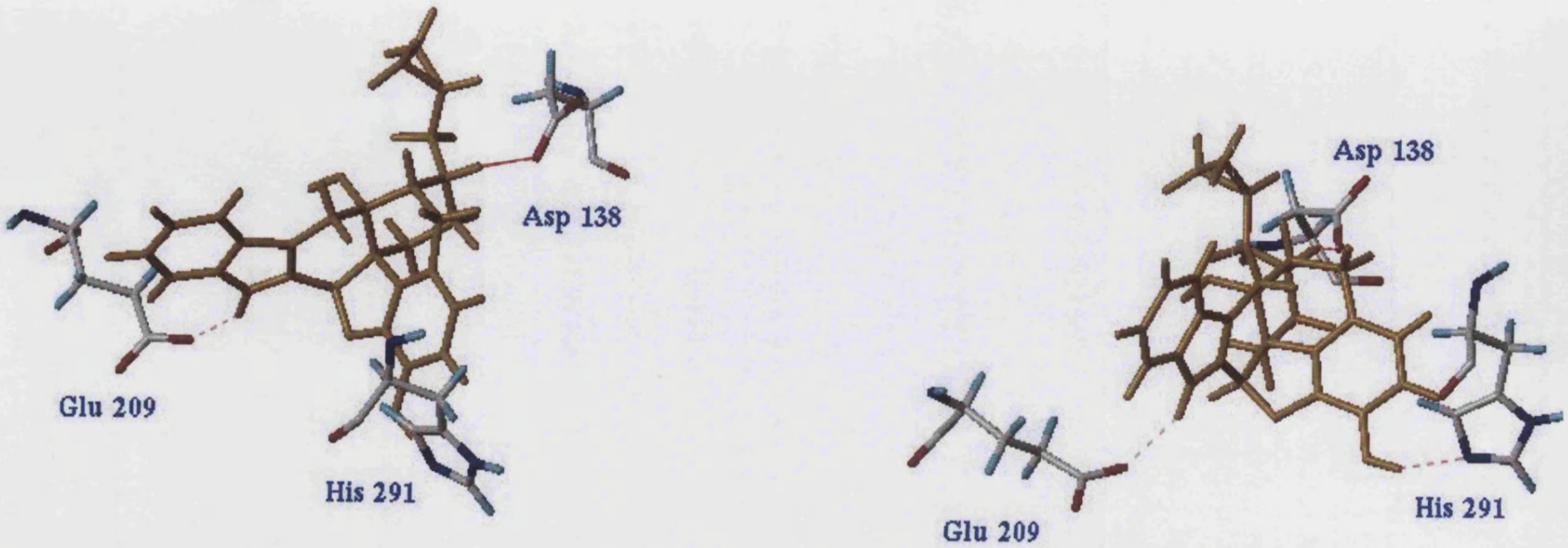


Fig. 5 Ionic interactions and hydrogen bonds between naltrindole and the κ-opioid receptor

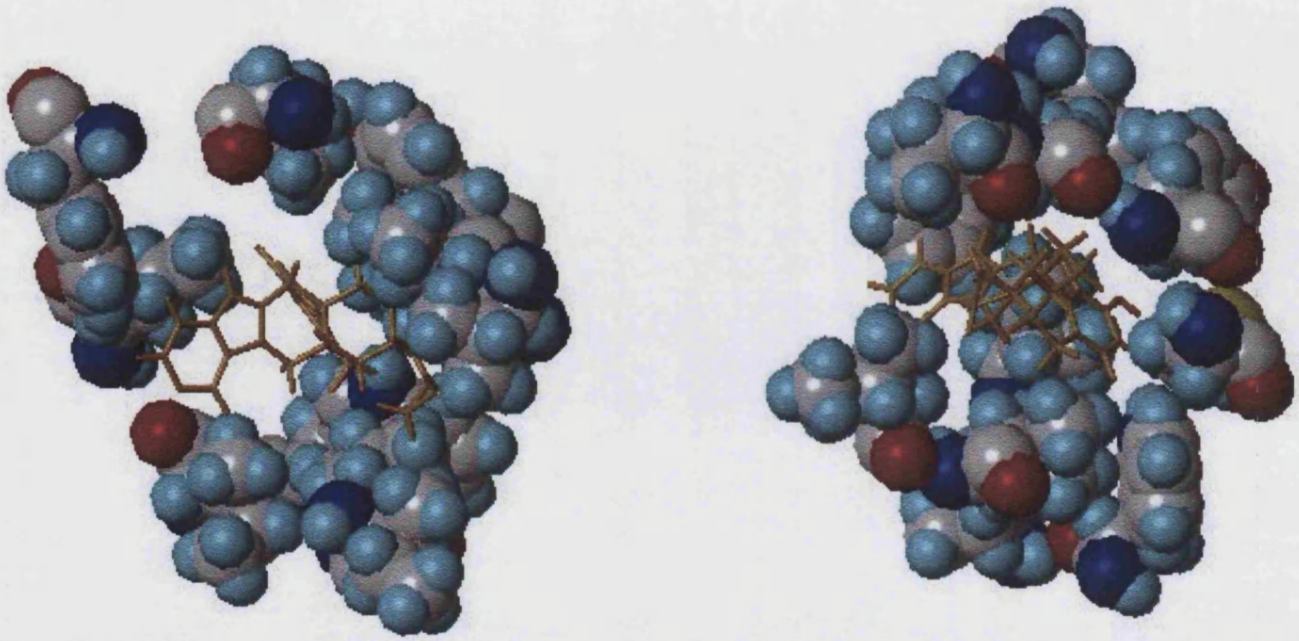
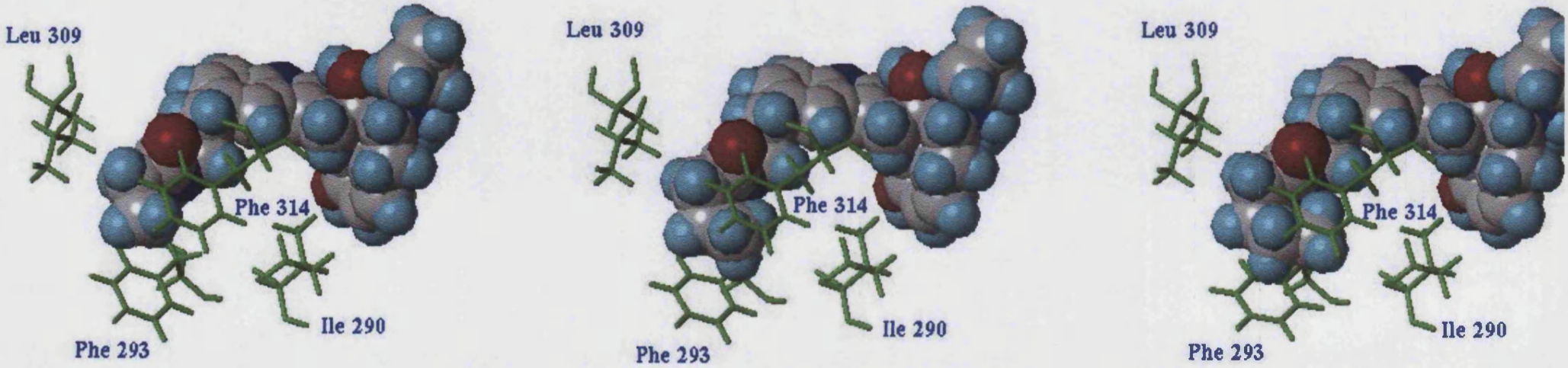


Fig. 6 Hydrophobic interactions between naltrindole and the κ-opioid receptor



130 131 132

Fig. 7 Hydrophobic interactions between alkyl amides (**130-132**) and the κ-receptor

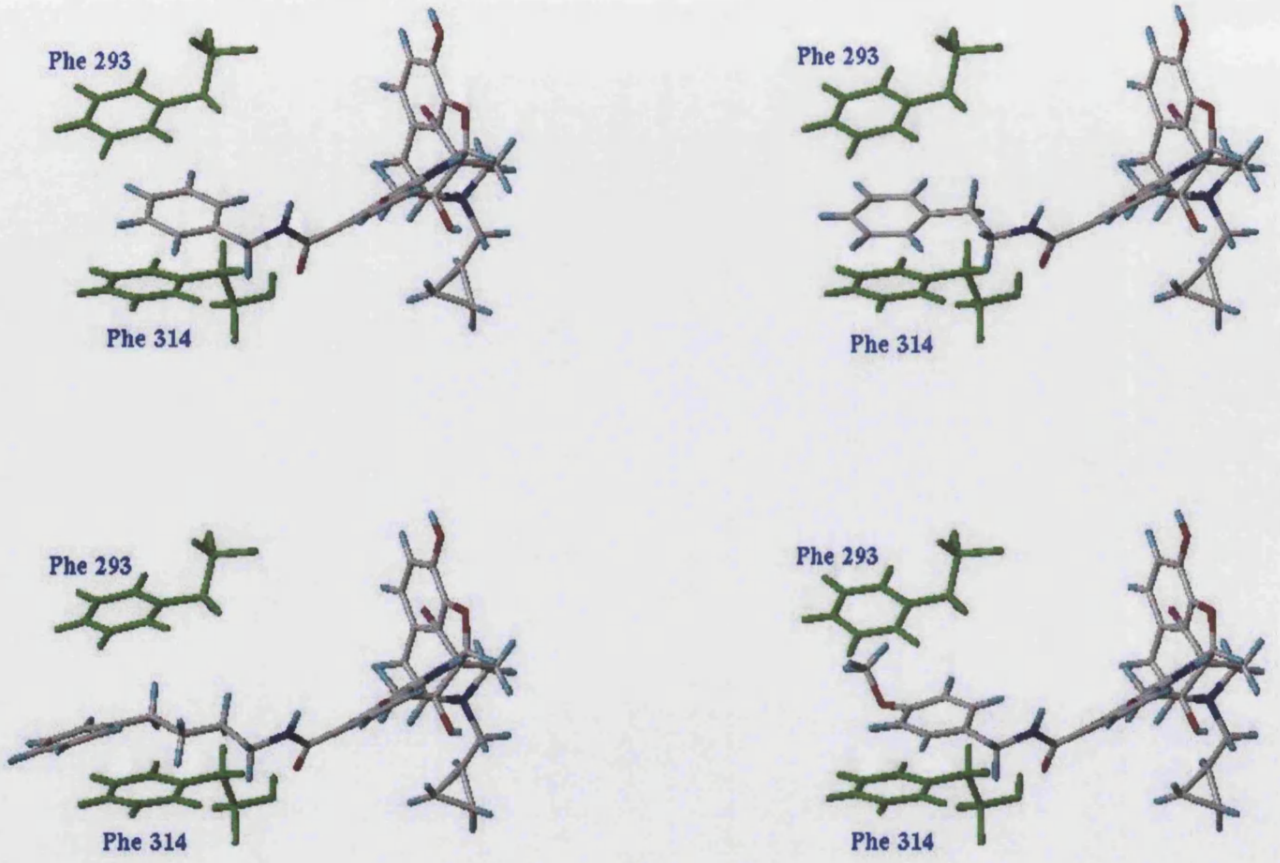


Fig 8 Hydrophobic interactions between aromatic amides (**60-63**) and the κ-receptor (clockwise from top left **60**, **61**,**63**,**62**)

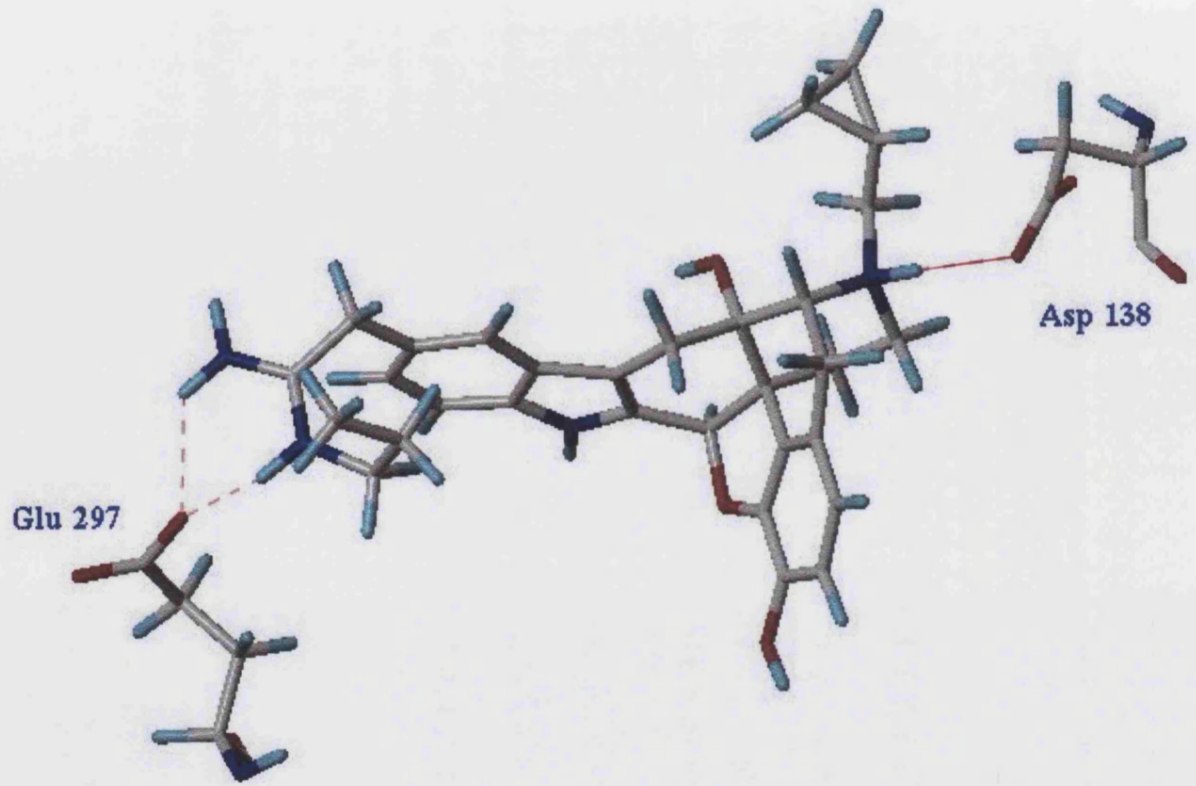
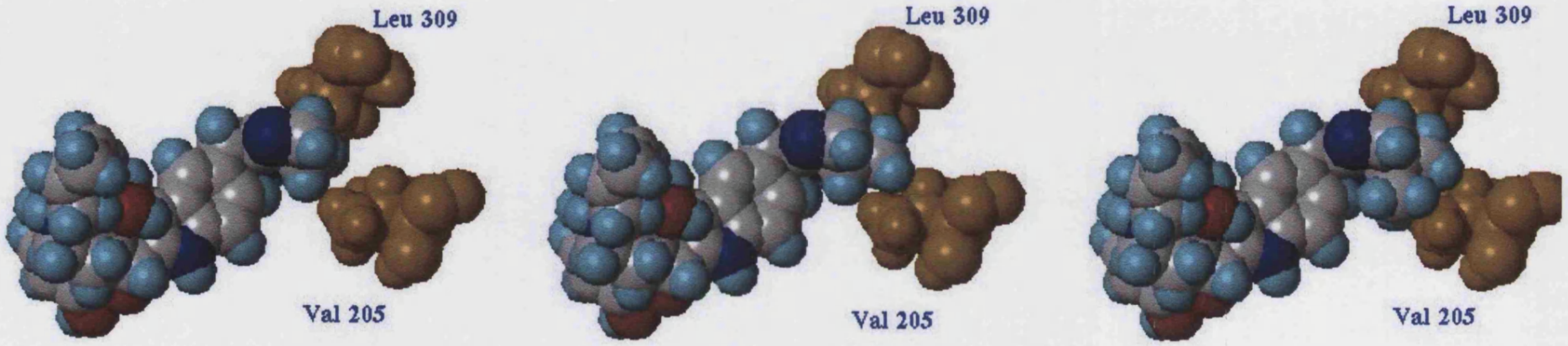
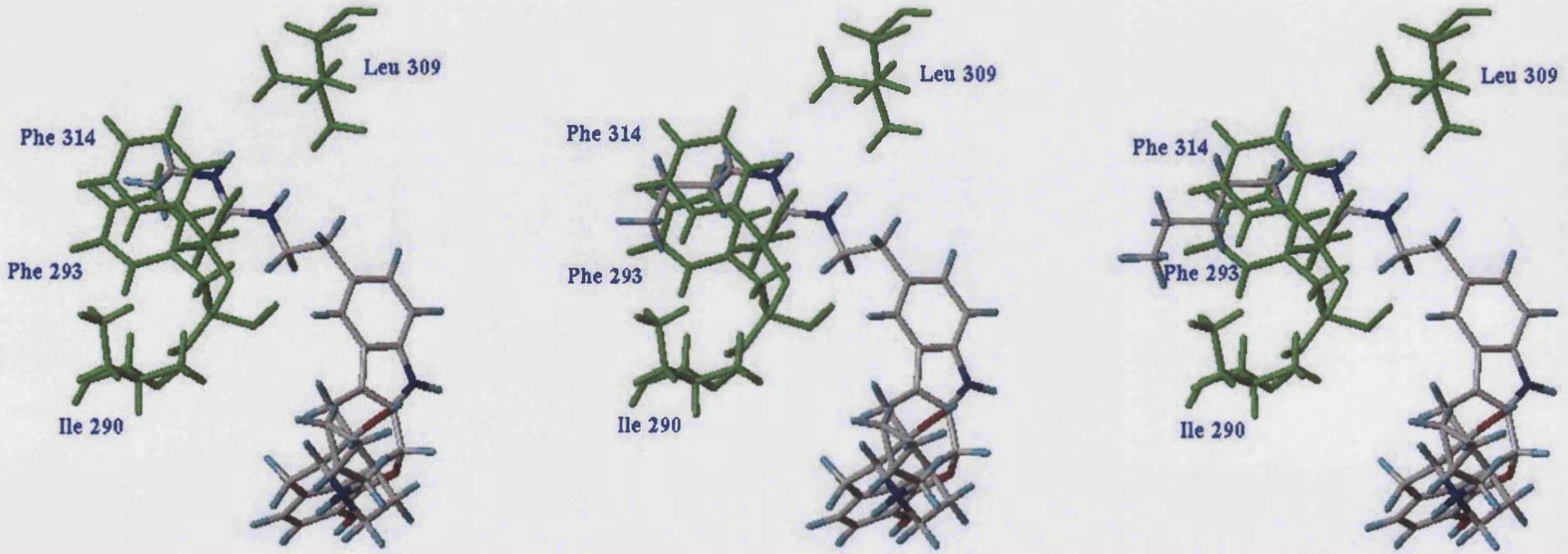


Fig.10 Ionic interactions and H-bonds between propyl reverse amidine (**72**) and the κ-receptor



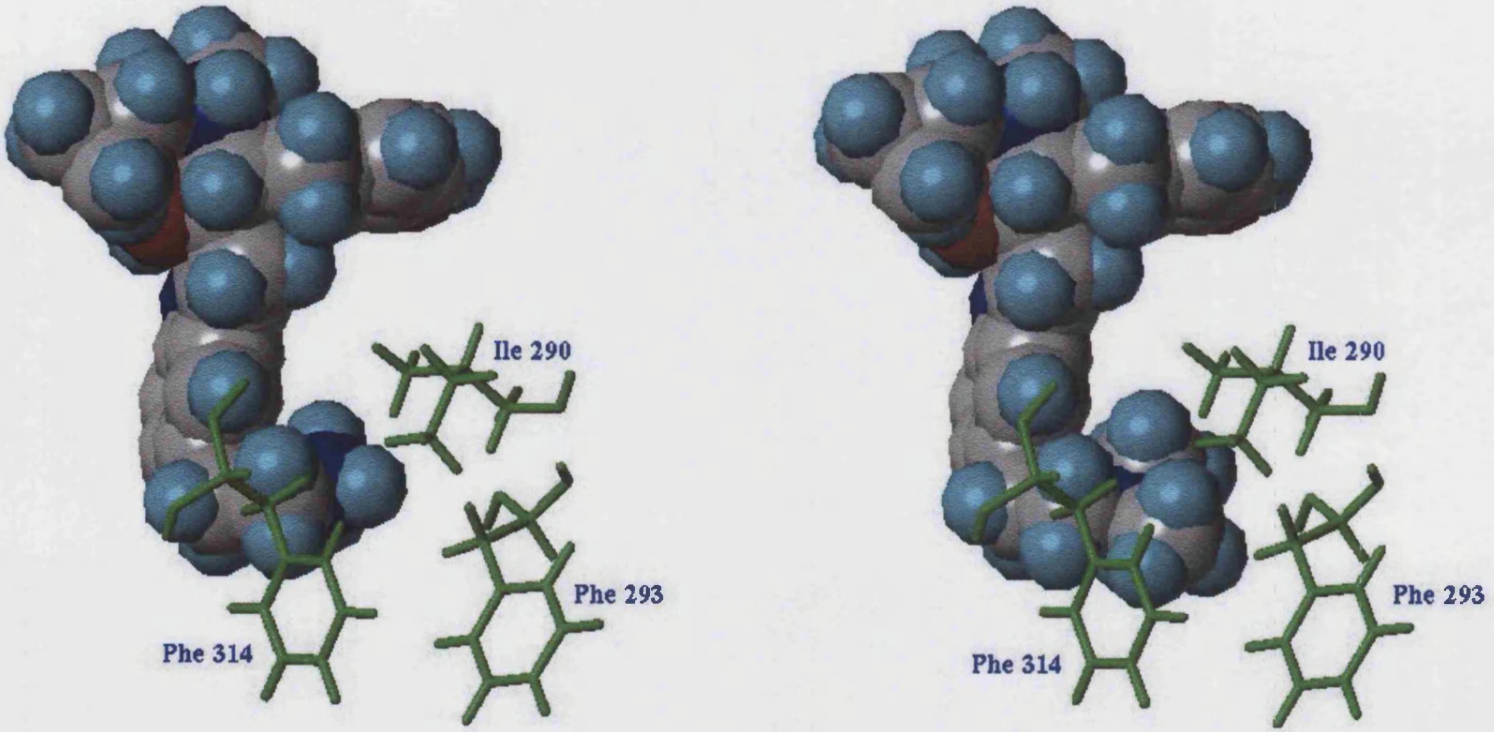
82 **83** **84**

Fig. 11 Hydrophobic interactions between imidazolines (**82-84**) and the κ-receptor



85 **86** **87**

Fig. 12 The unfavourable interaction of Leu 309 with the NH group of ureas (**85-87**)



76 **98**

Fig. 13 Hydrophobic interactions between amines (**76**, **98**) and the κ-receptor

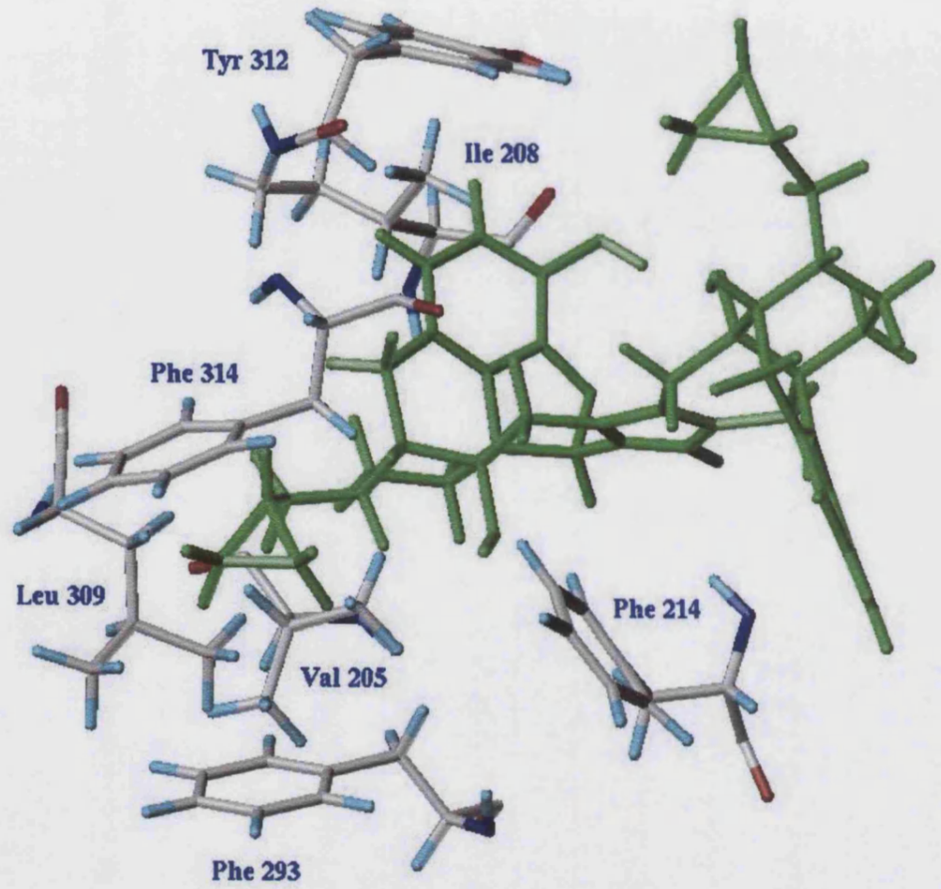


Fig. 17 Hydrophobic interactions between norBNI (**40**) and the κ-receptor

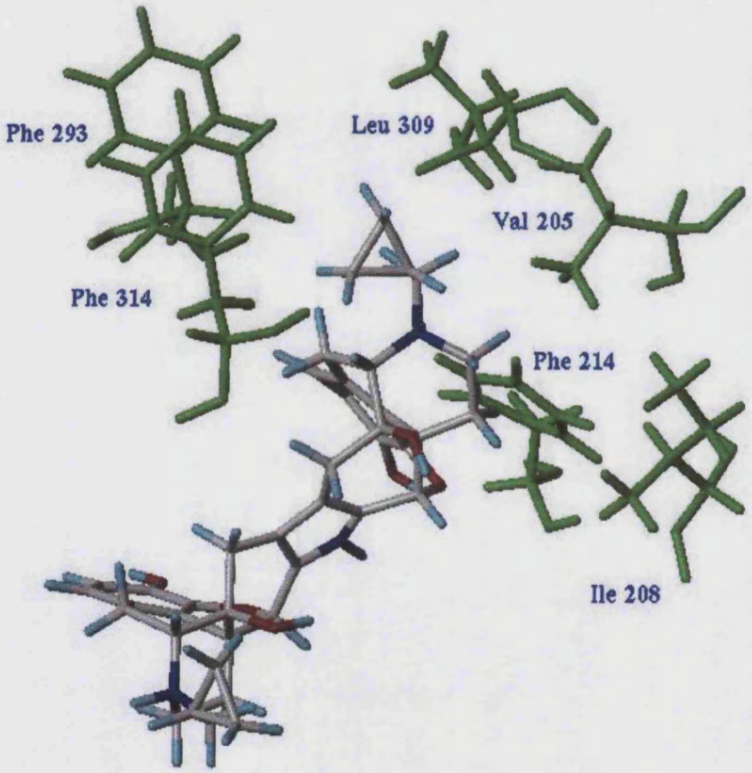


Fig. 18

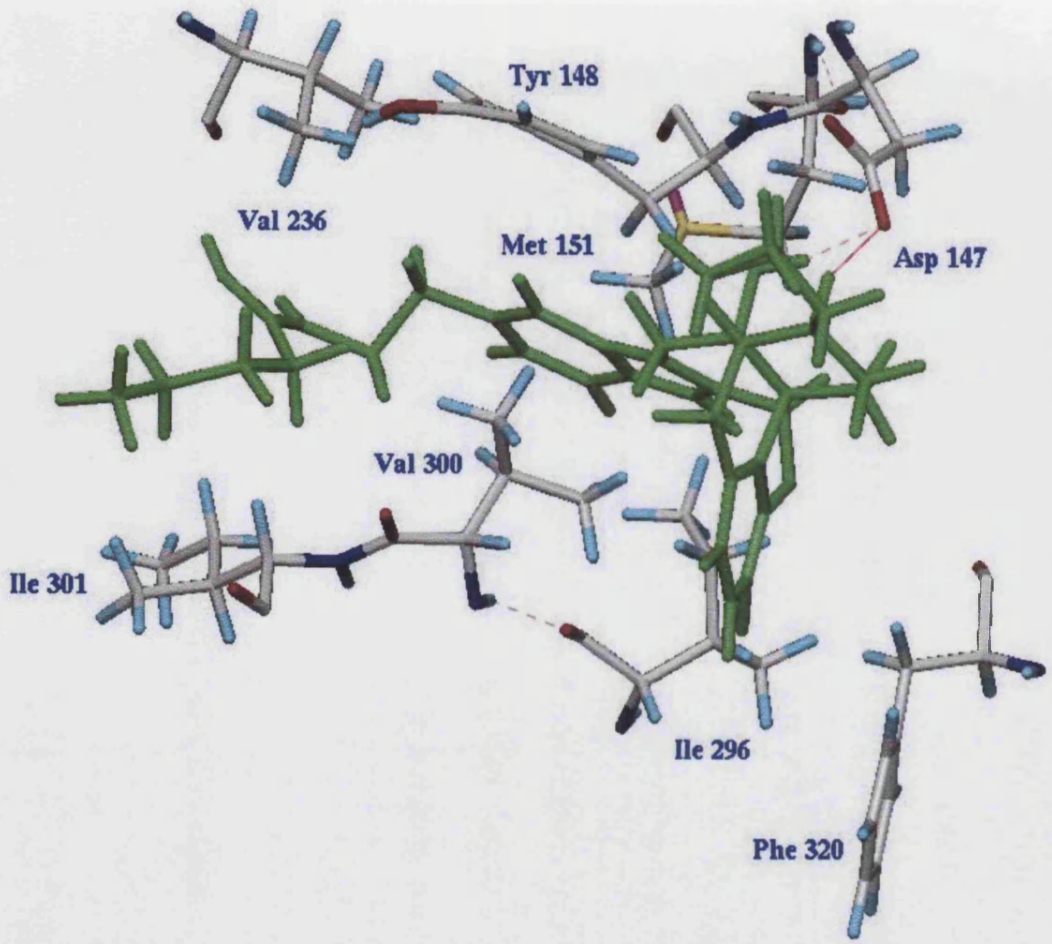


Fig. 23 Ionic interactions and H-bonds between urea (**87**) and the μ-receptor

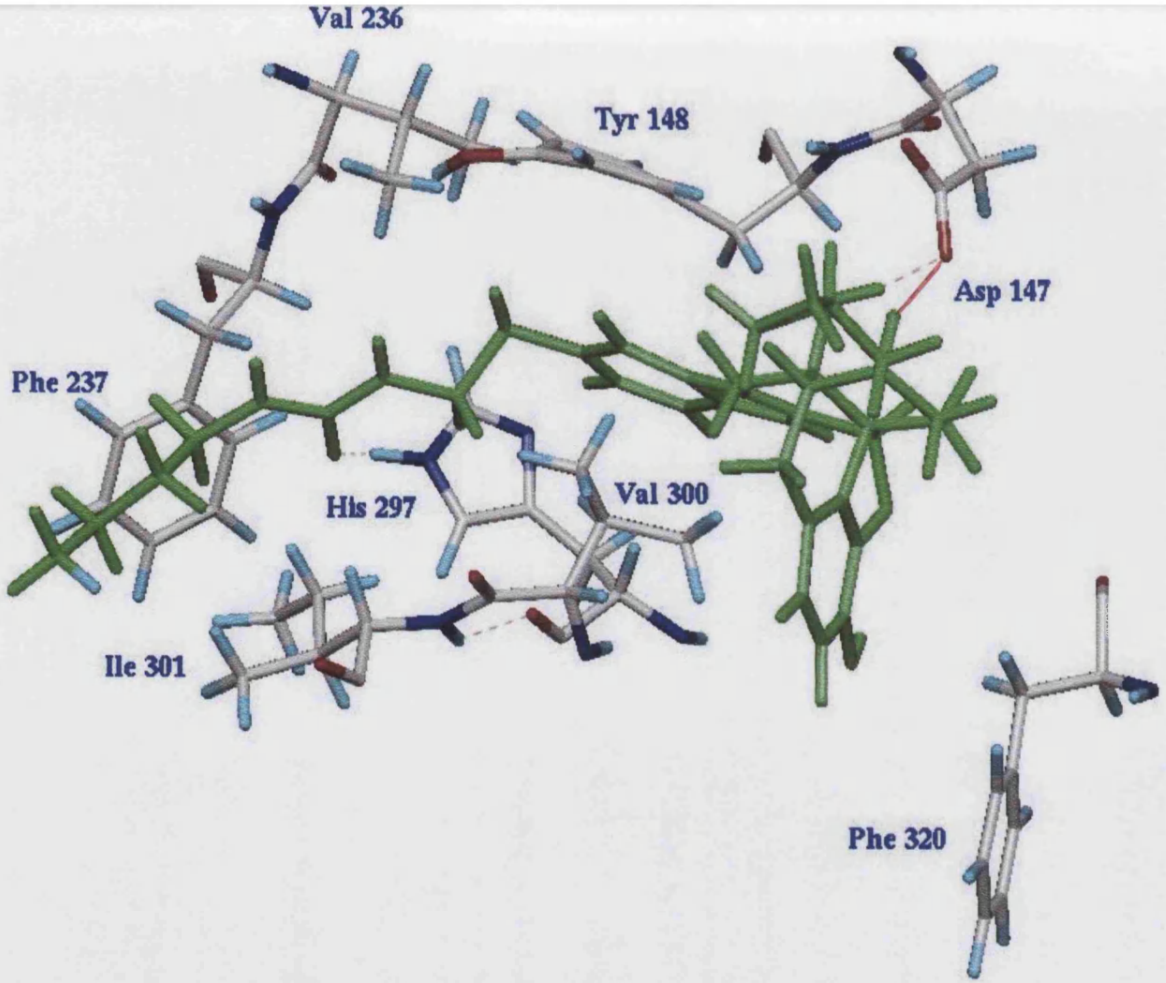


Fig. 25 Ionic interactions and H-bonds between urea (**86**) and the μ-receptor